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POST-EXERTIONAL ORTHOSTATIC HYPOTENSION

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Transitory non-fatal collapse following severe physical effort is a familiar phenomenon in competitive sport. Jokl[7] has described the clinical picture of this "Sportkrankheit" and Mateef and Petroff[8] and Mateef[9] first described and studied the orthostatic hypotension which is one of its circulatory aspects. Brogdon and Hellebrandt,[2] Eichna and Bean,[3] Mayerson,[10] Allen, Taylor and Hall[1] have confirmed the occurrence of orthostatic hypotension following hard muscular work. However, observations dealing with the nature of this postural hypotension are still few. While studying the physiologic effects induced in healthy young men worked to the limits of their physical tolerance, we encountered post-exertional orthostatic hypotension and had an opportunity to study it. Some of these studies are here presented.

Subjects and Methods. The subjects were all young, healthy soldiers (enlisted men) undergoing some phase of military training beyond basic training. They were, therefore, physically more fit than the usual normal group drawn from civilian life. The required work was performed at all hours of the day, except immediately after meals, and consisted of exercise tests designed to determine physical fitness. Two of the tests, the pack test and the treadmill test, were of the acute, exhausting type; the third, the hike, was of the long, enduring type. In the pack test,[5] the subjects stripped to shorts, socks and shoes, and carrying a pack weighing one-third of their body weight, stepped up and down on a platform 16 inches high, once every 2 seconds for 5 minutes, unless exhaustion forced them to discontinue before that time. In the treadmill test,[6] the subjects, similarly clothed but without pack, ran on a motor-driven treadmill for 5 minutes, unless forced by exhaustion to discontinue before that time. The speed of the treadmill was 7.5 miles per hour for the initial tests and 5.6 miles per hour for the repeat tests; in all tests the treadmill grade was 8.6%. The physical effort of these 2 tests is such that approximately one-third to one-half of the healthy young men attempting them fail to complete the required 5 minutes of work. In the hike, the men wore regulation fatigue uniforms of herringbone twill, carried packs weighing 20 pounds and were required to walk 32 miles in the best time they could, usually 7 to 9 hours. The pack and treadmill tests were performed indoors at an ambient temperature of about

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74° F. The hike tests were performed on the open road in the moderately warm weather of late spring. On completing the pack and treadmill tests, the men sat down for 5 minutes before postural studies were begun. In the hike tests these studies were started immediately after finishing the march.

Except after several of the hikes, when men changed from the lying to erect (90 degree) position by their own effort, position was always changed passively by means of a tilt table. The erect position was 70 degrees erect with the weight supported by the legs. No attempt was made to diminish postural sway or movement, other than repeated admonitions to stand still. Position was changed alternately from erect (70 degrees) to flat and flat to erect (70 degrees) at 5 minute intervals, except when syncope shortened the time in the erect position. While in each position the heart rate (by palpation or auscultation) and blood pressure (by auscultation, using a mercury manometer) were simultaneously and repeatedly determined, usually at 30 to 60 second intervals. At least one set of determinations in the flat and erect (70 degree) positions preceded an exercise test. Following exercise, all subjects were observed through at least 2 erect periods, separated by 1 in the supine, and thereafter through as many changes of position as the individual case required. In certain instances respiratory rates, vital capacities, and electrocardiograms were taken in each position before and after exercise.

In a number of subjects the relationship between their postural responses after exercise and their cardiovascular lability was studied by determining the changes in heart rate and blood pressure induced by several stimuli administered in the control period: (*a*) posture test, change from supine to erect (70 degrees); (*b*) cold pressor test, immersing one forearm in ice water for 2 minutes; (*c*) mental pressor test, requiring the subject to do arithmetical problems (multiply 3 digits by 2 digits) in his head for 5 minutes; (*d*) exercise test, changes during the 5 minutes immediately on completion of standard exercise.

Results. The post-exertional orthostatic responses are divided into 3 groups: syncopal, abnormal and normal. The *syncopal* group includes those subjects who developed syncope and were unable to remain erect for 5 minutes during either one or both of the 2 required erect periods after exercise. While erect, their blood pressures were usually very low and their heart rates rapid but collapse rather than any level of blood pressure or heart rate was the criterion for inclusion in this category. When upright, these subjects developed the typical symptoms and signs of syncope, either singly or in varying combinations, and in varying intensity. Almost invariably present were marked fatigue, drowsiness, apprehensiveness, increasing discomfort, nausea, abdominal cramps, lightheadedness and dizziness, and the sensation of impending collapse. In the more severe instances, dimness of vision progressing to tubular vision and "blackout," vomiting, disorientation, inability to move or obey commands even though hearing them progressed to complete loss of consciousness and crumpling at the knees, at which time the men were tilted flat. These symptoms were accompanied by various signs, yawning, deep breathing, increasing pallor leading to a pallid ashen cyanosis, profuse sweating, gasping respiration, apprehensive restlessness or listlessness, a steadily falling blood pressure with very narrow pulse pressure, markedly decreased intensity of heart sounds, and a rapid, weak pulse. Just before consciousness was lost, and the subject tilted supine, the heart in a number of subjects slowed markedly. Once supine, all symptoms quickly improved or disappeared completely. For a short time immediately after becoming supine, a marked bradycardia and elevation of blood pressure were often encountered, but as the supine position was maintained both heart rate and blood pressure returned to normal.

The *abnormal* group includes those subjects who were able to remain erect for the required 5 minutes during both of the post-exertional erect periods, but who, during at least 1 of them, sustained a fall in systolic blood pressure to abnormally

low levels, here defined as 100 mm. Hg or less, provided that that level was at least 10 mm. Hg lower than the lowest pre-exercise, erect, systolic blood pressure. Many men developed symptoms and signs similar to those in the syncopal group. Usually they were more mild but in some instances they were so severe that the men were just able to remain erect for the required 5 minutes. If the erect period had been further prolonged some men would surely have fainted. Others had no symptoms though severely hypotensive.

The *normal* group includes all of the others, men who remained erect after exercise without symptoms and with systolic blood pressures above 100 mm. Hg.

TABLE 1.—BLOOD PRESSURE, HEART RATE AND DURATION OF ERECT POSTURE AFTER ACUTE EXHAUSTING WORK

(The data for each group are averages of the final readings taken before position of subject was changed)

		First erect period					Supine period (5 min.)				Second erect period				
			Blood pressure (mm. Hg)				Blood pressure (mm. Hg)					Blood pressure (mm Hg)			
Group	No. subjects	Duration (min.)	Syst.	Diast.	Pulse	Heart rate (per min.)	Syst.	Diast.	Pulse	Heart rate (per min.)	Duration (min.)	Syst.	Diast.	Pulse	Heart rate (per min.)
Normal	14	5.0	119	88	31	115	117	73	44	100	5.0	114	90	24	112
Abnormal	10	5.0	103	76	27	116	117	67	50	105	5.0	95	83	12	116
Syncopal	9	2.4	*98	*72	*26	*122	118	68	50	108	2.6	‡81	†64	†17	118

* Average of the 4 subjects with obtainable blood pressure; blood pressure unobtainable in 5 subjects.
† Average of the 5 subjects with obtainable blood pressure; blood pressure unobtainable in 4 subjects.
‡ Average of the 6 subjects with obtainable blood pressure; blood pressure unobtainable in 3 subjects.
* Average of 8 subjects; pulse missing on 1 subject.

ORTHOSTATIC HYPOTENSION FOLLOWING ACUTE EXHAUSTING PHYSICAL WORK. Since the pack test and treadmill test both required essentially the same type of physical effort and produced very similar findings, the results of these 2 tests are combined into one analysis.

Type of Response and Incidence. Of 33 men subjected to acute exhausting physical work approximately one-half (19 or 57.6%) developed post-exertional orthostatic hypotension, with 9 (27.3%) in the syncopal group and 10 (30.3%) in the abnormal group (Table 1). Thus, every other man developed post-exertional orthostatic hypotension and 1 man in 4 had syncope. Table 1 indicates the progressive fall in blood pressure from the normal through the abnormal to the syncopal group, and for the same group order an increasing, but less striking, rise in heart rate. Syncope was most closely related to the systolic blood pressure and was most likely to occur when it fell to about 80 mm. Hg. Pulse pressure bore less relationship to syncope: the pulse pressure for the abnormal group was as low, and even lower, than that for the syncopal group (second erect period, Table 1). Circulatory failure in the erect position was more marked, as a rule, in the second erect period, 15 to 20 minutes after the cessation of exercise, than in the first erect period, 5 to 10 minutes after work. This is indicated by the greater number of syncopes and the greater fall in blood pressure in the second erect period than in the first (Table 1). The similarity in blood pressures for all 3 groups when the subjects were supine indicates that the circulatory disturbance is apparent only in the erect position.

Chart 1 indicates the response of a man in the normal group. Charts 2 and 3 are typical of 2 types of syncopal responses. In Chart 2 the hypotension and syncope develop "immediately," in the first erect period, but nevertheless, become more severe in subsequent upright periods. In Chart 3 the onset of hypotension and syncope are "delayed." The first erect period is tolerated without difficulty and with sustained blood pressures and heart rates. Hypotension and syncope develop in this second erect period and remain

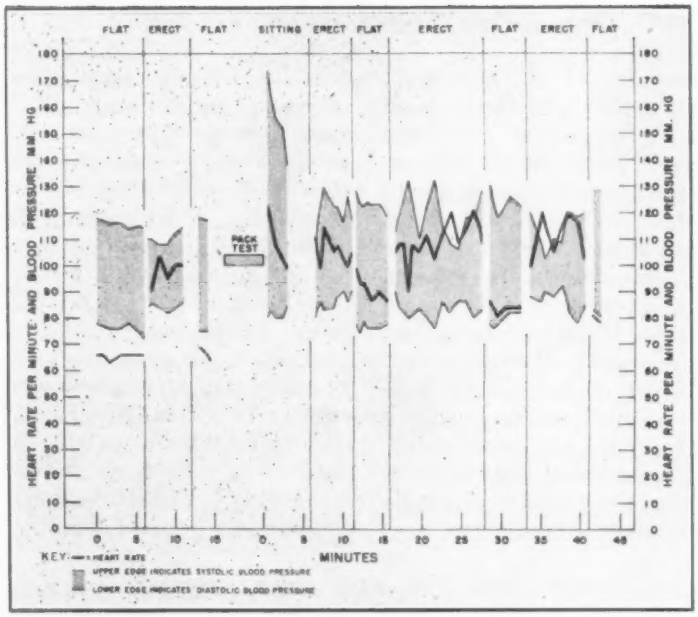

CHART 1.—Normal blood pressure in the erect posture following acute exhausting exercise. In this and subsequent charts the heavy black line indicates the heart rate; the upper level of the shaded area the systolic blood pressure; the lower level of the shaded area the diastolic blood pressure.

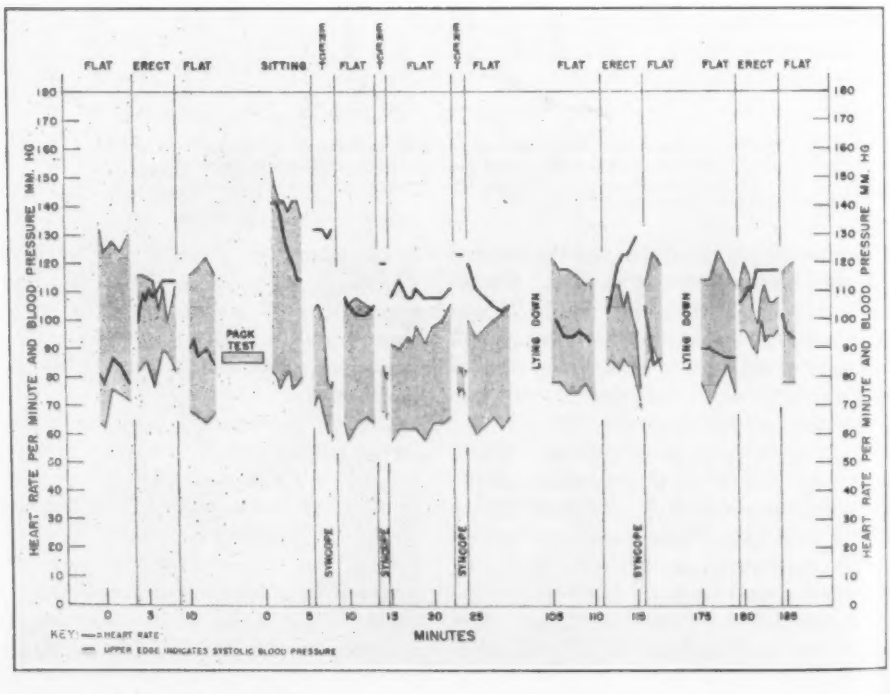

CHART 2.—Orthostatic hypotension with syncope following acute exhausting exercise. The hypotension and syncope develop during the first erect period after exercise and are still present 2 hours after cessation of effort.

severe thereafter. Usually tachycardia accompanies the hypotension (Chart 2) but at times the heart slows just as collapse is about to occur (Chart 3). In 1 instance (Chart 3 at point X) such a cardiac slowing progressed to an asystole of 19 seconds, with collapse of the subject.[4]

sures. In another subject (Chart 2) orthostatic syncope still occurred almost 2 hours after the exercise. Three hours after work, the erect posture was tolerated for 5 minutes, but the blood pressure was not yet normal. As a rule, there was recovery from syncope within 1 hour and from

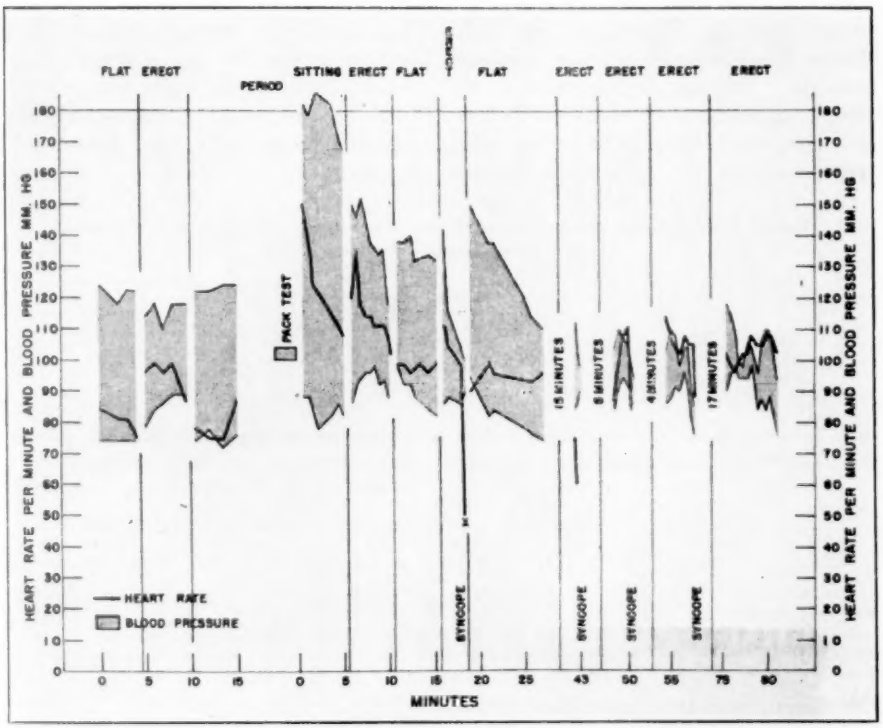

CHART 3.—Orthostatic hypotension with syncope following acute exhausting exercise. The hypotension and syncope are delayed in appearance and do not develop until the second erect period, following a first erect period which was tolerated without difficulty. Terminal bradycardia accompanied the hypotensive episodes.

Duration. The duration of the post-exertional orthostatic hypotension varies but it tends to persist for an unexpectedly long time. Thus in 1 subject (Chart 3) orthostatic hypotension with syncope was still present 1 hour after stopping work and it was not until 1 hour and 15 minutes after the exertion that he could remain erect for the required 5 minutes. Even then the blood pressure had not yet returned to normal but fluctuated widely, with low systolic and narrow pulse pressures. In another subject (Chart 2) orthostatic syncope still occurred almost 2 hours after the exercise. Three hours after work, the erect posture was tolerated for 5 minutes, but the blood pressure was not yet normal. As a rule, there was recovery from syncope within 1 hour and from hypotension (abnormal group) within 2 hours. The progressive improvement of the orthostatic hypotension with time is indicated in Charts 2 and 3 by the increasing length of the successive erect periods before the development of hypotension and syncope.

Type of Subject. The physical characteristics of the subjects and their cardiovascular responses to various stimuli gave little basis for predicting who would develop this syndrome. There were but

minimal differences in the physical characteristics of the subjects of the 3 groups (Table 2). The men in the syncopal group averaged slightly taller (3 inches) and heavier (12 pounds) than the men in the normal group. There were no significant differences in the ages of the 3 groups. Physical fitness, as determined by fitness tests, was essentially the same in all 3 groups (Table 2). There were only slight differences in the cardiovascular responses to stimuli. Perhaps the responses in the syncopal group may be considered somewhat more labile than those in the other 2 groups (Table 3). The most marked differences lay in the higher pulse rates immediately after acute exhausting work and in the greater pressor response to the cold test in the syncopal group.

Thus, of 5 subjects who were in the syncopal group after the first test, only 1 remained in the syncopal group following a repeat test; 2 moved into the abnormal group and 2 into the normal group. Similarly, of 7 subjects in the abnormal group after the first test, only 3 remained in the abnormal group following a repeat test, while 4 moved into the normal group. Ten men in the normal group on the first test remained in the normal group on repeat tests. In all instances the physical fitness scores were not significantly different on repeat tests from those on the initial tests.

TABLE 2.—PHYSICAL CHARACTERISTICS AND PHYSICAL FITNESS OF THE SUBJECTS
(Data are the average for each group)

		Physical characteristics			Physical fitness	
Group	No. subjects	Age (yrs.)	Height (ft., in.)	Weight (lbs.)	Time of effort (min.)	Fitness score
Normal	14	26	5′6″	152	3.5	59
Abnormal	10	22	5′6″	158	3.4	52
Syncopal	9	23	5′9″	164	3.8	52

TABLE 3.—COMPARISON OF THE CARDIOVASCULAR RESPONSES OF THE THREE GROUPS TO STIMULI
(The data for each group are averages of the most marked response during the stimulus)

		Supine				Erect (70°)				Cold pressor test*				Mental problem†				Exercise‡			
		Blood pressure (mm. Hg)				Blood pressure (mm. Hg)				Blood pressure (mm. Hg)				Blood pressure (mm. Hg)				Blood pressure (mm. Hg)			
Group	No. subjects	Syst.	Diast.	Pulse	Heart rate (per min.)	Syst.	Diast.	Pulse	Heart rate (per min.)	Syst.	Diast.	Pulse	Heart rate (per min.)	Syst.	Diast.	Pulse	Heart rate (per min.)	Syst.	Diast.	Pulse	Heart rate (per min.)
Normal	14	119	73	46	71	118	88	30	91	134	91	43	71	135	92	43	89	166	78	88	145
Abnormal	10	117	73	44	71	112	85	27	94	134	92	42	74	129	86	43	86	149	69	80	166
Syncopal	9	122	73	49	77	115	86	29	101	141	98	43	70	136	88	48	67	158	75	83	172

* Two minutes of immersion of forearm in iced water.
† Five minutes of mentally multiplying 3 digits by 2 digits.
‡ Immediately on termination of pack test, subject seated.

Repeat Tests. With repetition of the work on subsequent days there is a tendency for improvement, and even disappearance, of the post-exertional orthostatic hypotension and syncope (Chart 4).

Mechanism of the Hypotension. Several simple observations indicated the major rôle played by the motionless, dependent lower extremities in the production of the hypotension.

With the trunk still erect, raising the lower extremities to a horizontal position, so that the heels were on the same level as the buttocks materially raised the blood pressure (Chart 5). This maneuver did not, however, return the blood pressure to normal. A definite hypotension (abnormal group) still persisted but it was not so severe as to cause syncope. Syncope, however, promptly recurred when the legs

were lowered to the dependent position (Chart 5). Apparently the distribution of blood in both the splanchnic area and in the legs is responsible for the orthostatic hypotension and dependency of the lower extremities is critical to the production of the full effect.

static hypotension promptly returned (Chart 6).

When blood is prevented from flowing into the lower extremities of the upright subject; orthostatic hypotension does not occur; the blood pressure is sustained and a previously syncopal subject remains

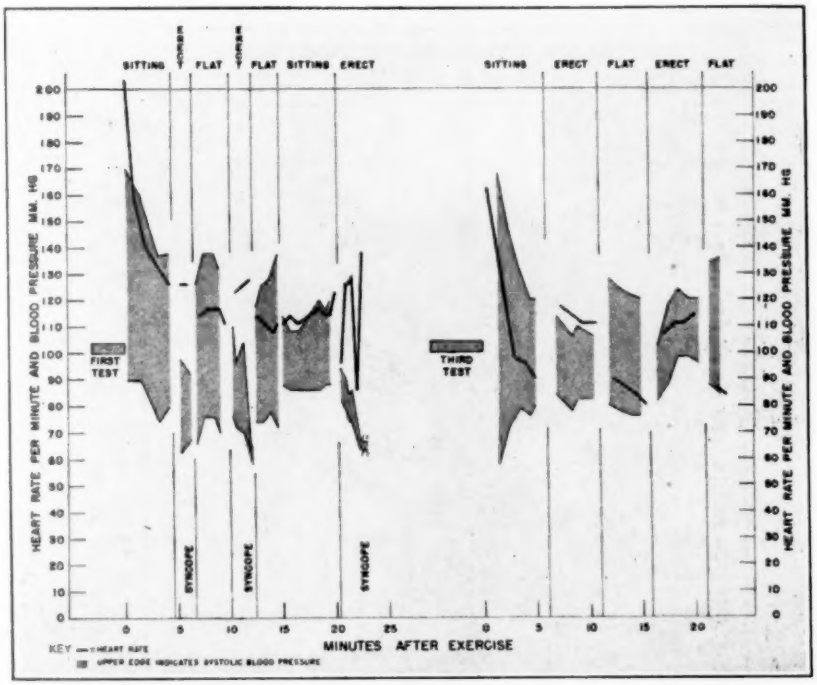

CHART 4.—Disappearance of post-exertional orthostatic hypotension with repetition of the inducing acute exhausting exercise. Marked orthostatic hypotension after the first test; normal blood pressure when erect after the third test.

During the orthostatic hypotension, movement of the fully dependent legs, without the accomplishment of much additional work, caused the blood pressure to rise from its low level to higher, and even normal, levels which were maintained as long as movement of the legs was continued (Chart 6). The movement of the legs here used consisted of bending the knee forward sufficiently for the heel to clear the foot board of the tilt table, the toe retaining contact with it. The legs were moved one at a time and alternately. With cessation of their movement orthoerect without difficulty (Chart 7). Just before the subject was tilted erect, the circulation to both legs was occluded by suddenly inflating, without venous stasis, blood pressure cuffs encircling both thighs. The cuffs were of standard 13 cm. width, backed by heavy cloth so that they did not balloon, and were inflated to 240 mm. Hg from pressure bottles. With the cuffs thus inflated orthostatic hypotension did not occur but it developed rapidly in preceding and subsequent erect periods when the thigh cuffs were not inflated (Chart 7).

Trapping as much blood as possible in

both lower extremities of a non-exercised subject failed to produce definite orthostatic hypotension, although some lowering of the systolic pressure and narrowing of the pulse pressure did occur (Chart 8). Reactive hyperemia was utilized to produce full dilatation of the vascular beds of the legs in which the blood was then trapped. With the subject flat, blood pressure cuffs about both upper thighs were inflated to 240 mm. Hg occluding the circulation to both legs. After 10 minutes the subject was tilted erect and 1 minute later both cuffs were deflated producing marked hyperemia of the dependent legs. In the first instance the cuffs were deflated to zero, permitting accumulation of blood in the dilated vascular beds of the legs under the influence of gravity alone; in the second instance they were deflated to 50 mm. Hg, trapping the blood under the influence of this resisting barrier as well as gravity. Chart 8 shows the marked drop in blood pressure immediately at the onset of reactive hyperemia and then the spontaneous and rapid recovery to a higher level, presumably as a result of vasoconstriction of other vascular areas of the body. The subject then remained erect without symptoms, in spite of the pooling in the legs of presumably as much

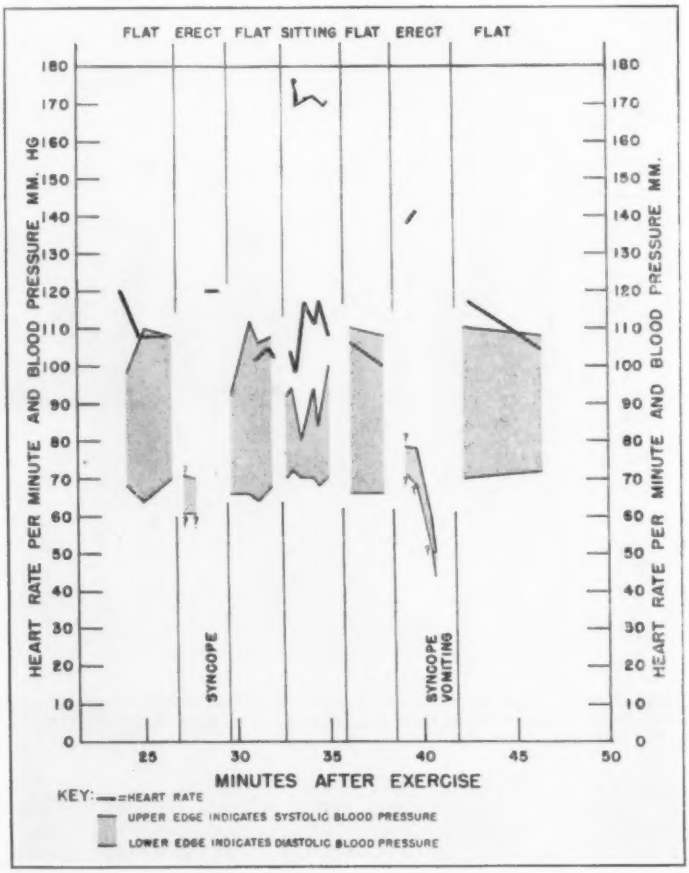

CHART 5.—Improvement in the post-exertional orthostatic hypotension when the legs are raised to a horizontal position at the level of the pelvis. (Indicated by diagram at top of sitting column.)

blood as their vascular beds would hold. This subject developed orthostatic hypotension following the pack test.

ORTHOSTATIC HYPOTENSION FOLLOWING PROLONGED ENDURING PHYSICAL EFFORT. Limited observations made after the completion of long-sustained physical work of lower energy expenditure indicated that post-exertional orthostatic hypotension occurs after this type of work as well as after acute exhausting physical effort. The 32 mile hike was attempted by 24 men; 22 finished, 2 dropped out after 28 miles. The responses of these 2 men are grouped with the others. Of the 24 men, 13 (54.2%) developed post-exertional orthostatic hypotension; 5 men (20.8%) were in the syncopal group and 8 men (33.3%) in the abnormal group (Table 4, Chart 9). This incidence is similar to that after acute exhausting exertion. After the hike test, the second erect period was omitted except in those subjects who developed hypotension during the first erect period and it is possible that late developing hypotensions were missed.

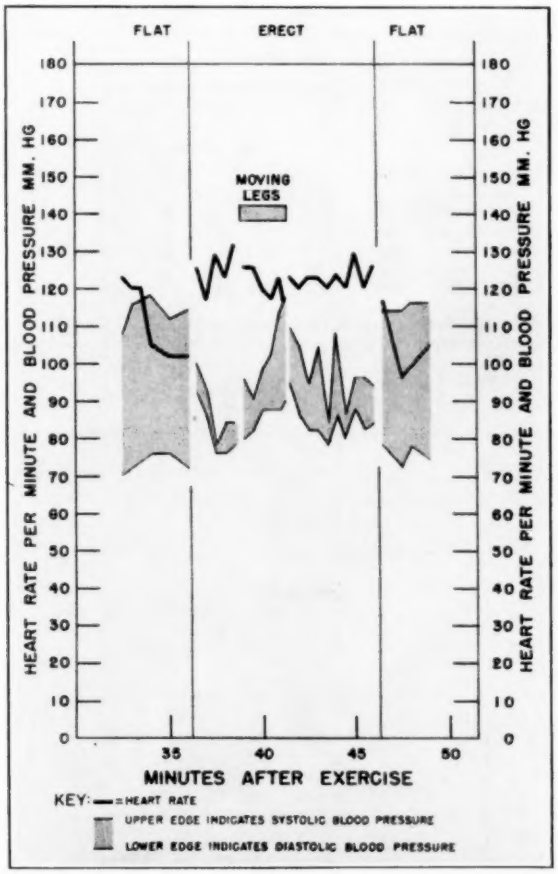

CHART 6.—Improvement in the post-exertional orthostatic hypotension when the dependent legs are moving.

The orthostatic hypotension induced by prolonged enduring work was very similar to that after acute exhausting work. Its development bore no relationship to the height, weight or age of the subjects

(Table 4) or to their physical fitness as determined by fitness tests and their general work performance. As after acute exhausting work, the orthostatic hypotension following the endurance hike often persisted for a long time. Usually the ability to remain erect, with a sustained blood pressure and without symptoms, returned in 1 to 2 hours after stopping work (Chart 9). In 1 instance, orthostatic hypotension was still present after a night's sleep, 12 hours after finishing the hike, and it was not until 16 hours after the hike that the erect posture could be main-

TABLE 4.—PHYSICAL CHARACTERISTICS OF THE SUBJECT GROUPS AND THEIR CARDIOVASCULAR RESPONSES AFTER 32 MILE HIKE

(The data for each group are averages of the final readings taken before position of subject was changed)

		Physical characteristics			First erect period					Second erect period				
						Blood pressure (mm. Hg)					Blood pressure (mm. Hg)			
Group	No. subjects	Age (yrs.)	Height (ft., in.)	Weight (lbs.)	Duration (min.)	Syst.	Diast.	Pulse	Heart rate (per min.)	Duration (min.)	Syst.	Diast.	Pulse	Heart rate (per min.)
Normal	11	23	5'8"	152	5.0	114	85	29	110					
Abnormal	8	22	5'8"	158	5.0	94	79	15	114					
Syncopal	5	25	5'7"	157	2.7	*92	*82	*10	‡120	2.0	†79	†68	†11	108

* Data for the 1 subject with obtainable blood pressure; blood pressure unobtainable in 4 subjects.

† Average of the 3 subjects with obtainable blood pressures; blood pressure unobtainable in 1 subject, and second erect period omitted on 1 subject.

‡ Average of 4 subjects; pulse missing on 1 subject.

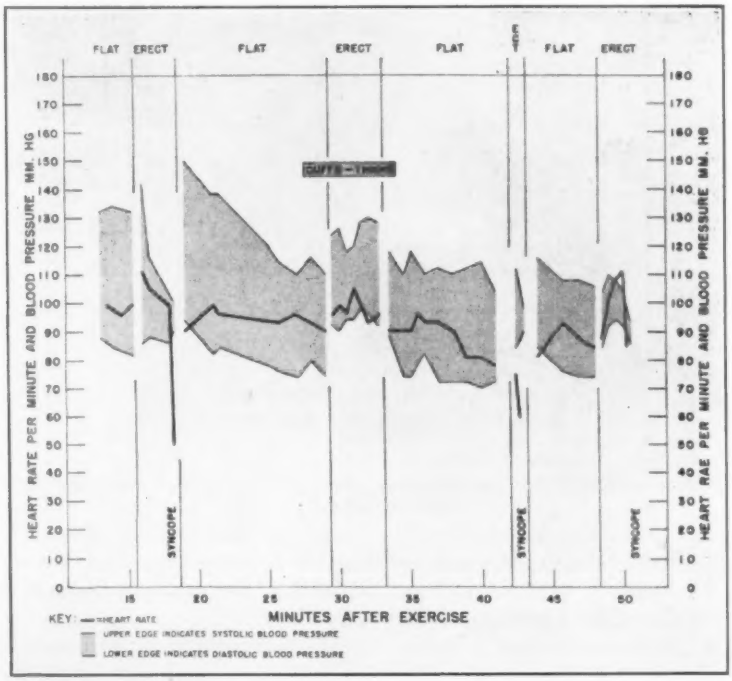

CHART 7.—Prevention of post-exertional orthostatic hypotension by excluding the circulation to the dependent legs. Just before the subject is tilted erect the cuffs about the thighs are inflated to 240 mm. Hg.

tained for 5 minutes. Even then the blood pressure was abnormal (Table 5). This subject also developed orthostatic hypotension after the pack test.

tered orthostatic syncope in 17 (17%) of 100 aviation cadets who had run to exhaustion on a treadmill. In all of these studies, including the present one, the

TABLE 5.—PERSISTENCE OF POST-EXERTIONAL ORTHOSTATIC HYPOTENSION IN A SUBJECT FOLLOWING AN ENDURANCE HIKE (32 MILES)

Time	Blood pressure subject erect (70°) (mm. Hg)	Heart rate (per min.)	Duration of erect posture	Remarks
Before hike	114/92	117	5 min.	No symptoms
1 hour after hike	Unobtainable	138	2 min.	Syncope
12 hours after hike (next morning)	Unobtainable	138	3 min.	Syncope
16 hours after hike	100/92	120	5 min.	Feels all right

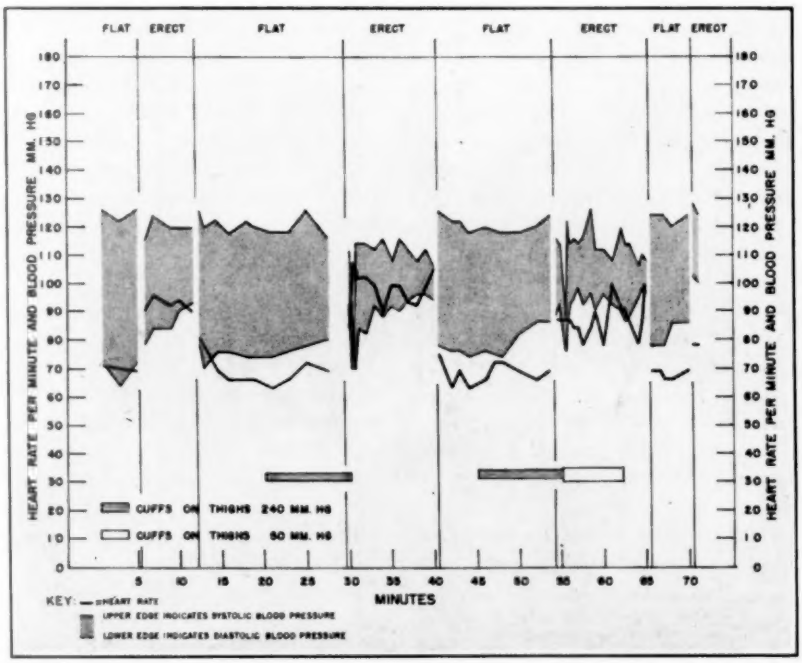

CHART 8.—Failure to produce orthostatic hypotension by trapping maximum amounts of blood in the dependent legs. Reactive hyperemia was used to induce maximal dilatation of the vascular beds of the legs and gravity plus venous occlusion to trap the blood in the dilated vessels.

Discussion. Combining the data following acute exhausting and prolonged enduring work into a group of 57 tests, post-exertional hypotension with syncope occurred in 14 (25%) and hypotension without syncope in 18 (32%). This agrees with the findings of other investigators. In a group of 50 students Mayerson[10] reported post-exertional orthostatic syncope in 17 (34%) and "poor response" in 10 (20%). Allen, Taylor and Hall[1] encountered orthostatic syncope in 17 (17%) of 100 aviation cadets who had run to exhaustion on a treadmill. In all of these studies, including the present one, the subjects have been healthy, young adult males, at times in especially good physical condition (athletes). One can only guess that the incidence in older age groups might be considerably higher, and the inducing physical effort less severe.

The erect position used in this study tends to minimize the incidence of orthostatic circulatory insufficiency: (*a*) the erect periods were maintained for only 5 minutes; others have required 10 to

20 minutes, and (*b*) the body weight was borne by the legs, permitting muscle tonus to aid in the venous return from the legs; others have eliminated this by supporting the body from the pelvis or shoulders. Since the erect man normally supports his weight on his feet, it was felt that observations performed in this manner would come nearer to reproducing the conditions under which this syndrome might occur in everyday life.

explanation an inadequate return of blood to the heart. The beneficial effects resulting from elevating the legs while the trunk remains erect, moving the legs when they are dependent, and excluding the circulation from the dependent legs, all suggest that the circulation in the lower extremities is at fault and responsible for the decreased venous return. The question is the nature of this circulatory fault.

Two possibilities present themselves:

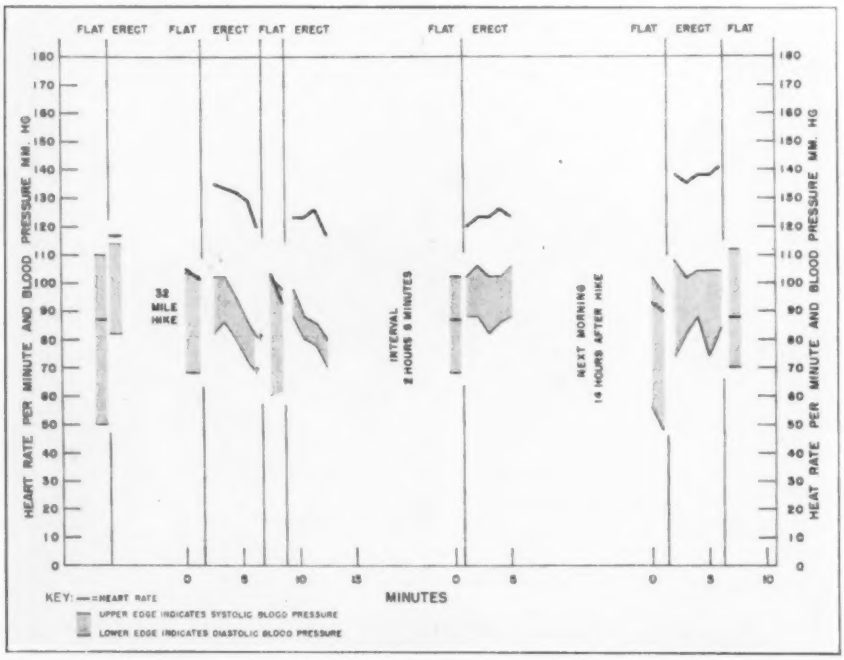
FLAT ERECT FLAT ERECT FLAT ERECT FLAT ERECT FLAT ERECT FLAT
HEART RATE PER MINUTE AND BLOOD PRESSURE MM. HG
32 MILE HIKE
INTERVAL 2 HOURS 6 MINUTES
NEXT MORNING 14 HOURS AFTER HIKE
MINUTES
KEY: HEART RATE
UPPER EDGE INDICATES SYSTOLIC BLOOD PRESSURE
LOWER EDGE INDICATES DIASTOLIC BLOOD PRESSURE

CHART 9.—Orthostatic hypotension with syncope following moderate work of long duration (32 mile hike).

The failure to establish a clear-cut relationship between post-exertional orthostatic hypotension and bodily configuration, tests of cardiovascular lability, or physical fitness gives, at present, no basis for predicting who will develop hypotension after physical effort. It can, however, be prevented by training, through repetition of the inducing physical work.

The present study does not explain completely the mechanism which permits the development of orthostatic hypotension after exertion, but suggests as a plausible (*a*) marked vasodilatation of the vascular tree of the legs and (*b*) failure of the muscular venopressor mechanism in the legs. The first of these assumes such a marked dilatation (exercise induced) of the vascular tree of the legs that the total vascular bed of the body is now disproportionately greater than the blood volume available to fill it at a normal pressure. The vascular tree of legs would contain an excessive amount of blood which would be flowing. The second possibility assumes that such a vasodilatation is not by itself capable

of producing the hypotension, as long as blood flow through the legs persists, and suggests that the blood in the legs has become stagnant through the failure of the muscular venopressor mechanism to move it onward and upward. The blood in the legs would be relatively non-moving. Since both mechanisms would reduce the amount of blood at heart level and induce hypotension, it may be considered an academic point to chose between them. Nevertheless, several observations suggest that failure of the venopressor mechanism may be a more critical factor than extensive vasodilatation. These are: (*a*) the delayed development of orthostatic hypotension in some subjects who stand without difficulty in the first erect period, when vasodilatation is greatest, and then develop hypotension in subsequent stands when vasodilatation is presumably diminishing (Chart 3); (*b*) the persistence of the orthostatic hypotension for long periods (several hours) after cessation of exertion, when the vascular dilatation may be considered to have largely passed off (Charts 2 and 3); (*c*) the recovery of the blood pressure from hypotensive toward normal levels when the dependent legs are moved without significant additional work (Chart 6); and (*d*) the failure to induce a similar hypotension and syncope when maximum amounts of blood are trapped in the dependent legs of erect but non-exercised subjects, who develop orthostatic hypotension after exercise (Chart 8). In this last instance, the large blood mass in the legs is presumably still moved forward by the unaltered muscular venopressor mechanism of non-exercised legs. While the above observations suggest that a reduced muscular tonus with a depressed venopressor mechanism in the legs plays the major rôle in the hypotension, this point has not been substantiated by direct experimentation. Neither has the degree of vasodilatation in the legs been measured.

This study, and similar ones in the literature, have all dealt with work performed either largely or wholly by the lower extremities. The critical, parallel observations following work of the upper extremities alone have yet to be carried out.

It is desirable to point out again that post-exertional orthostatic hypotension is not limited to acute exhausting effort and that it occurs with almost equal frequency, and with similar physiologic changes, after prolonged effort at a lower work rate. This, together with the striking persistence of the orthostatic hypotension for long periods after cessation of work may be of significance to clinical medicine in an understanding of the collapse states, and even death, which are encountered after physical effort.[4] If healthy, young men can develop circulatory failure while erect after exercise, one may guess that similar circulatory changes are not only likely in the older age groups, but that they may be more readily induced, more severe in their manifestations and capable of serious consequences. The effects of physical effort in the older age groups, and the relationship of the changes thereby induced to subsequent disability appears to be a fertile field for study.

Summary. 1. Orthostatic hypotension developed in approximately one-half of normal young men following vigorous exercise of the lower extremities. It followed prolonged moderate work as well as acute exhausting effort.

2. In one-half of those who developed orthostatic hypotension (one-fourth of all subjects) the hypotension was so severe that syncope resulted.

3. The orthostatic hypotension often persists for long periods, over 1 hour, after cessation of the inducing physical effort.

4. The causative factor appears to be a pooling of blood in the dependent lower extremities, presumably due to failure of the muscular venopressor mechanism in the legs, plus a work induced dilatation of their vascular beds.

5. During the orthostatic hypotension, maneuvers which move blood out of the lower extremities, or exclude blood from them, relieve the hypotension.

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THE TYROSINASE INHIBITING ACTION OF SERUM FROM NORMAL AND CANCEROUS PATIENTS*

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In a recent paper, Duboff and Hirshfeld[1] reported studies on the tyrosinase-inhibiting action of the serum of cancer patients. Using a system of crude potato tyrosinase, 0.001 M l-tyrosine, and 0.5 ml. of serum in a phosphate buffer, they determined the rate of color development. They reported that 89% of 140 cases of proven active malignant neoplastic disease showed an inhibition of color development equal to or greater than 15% when compared to controls without serum. In 117 normal subjects only 3, and in 68 cases with miscellaneous clinical conditions other than known cancer only 9 caused an inhibition greater than 15%.

On the basis of these findings, Duboff and Hirshfeld concluded that an inhibition greater than 15% in color development of their tyrosinase system is abnormal, and they discussed the possibility of using the inhibition of tyrosinase activity as a basis for a serodiagnostic test for cancer. In view of its obvious importance, the subject was studied in this laboratory using techniques which, so far as we are aware, were precisely similar (with minor exceptions which are noted) to the techniques used by Duboff and Hirshfeld.

Chemistry of Tyrosinase Action. The reactions of tyrosine in the presence of tyrosinase have been studied by Raper *et al.*[2,3] and recently reviewed by Nelson and Dawson.[6] Tyrosine is first oxidized to dihydroxyphenylalanine (DOPA). This step is not reversible. The colorless DOPA is then oxidized to DOPA-quinone. Reducing substances such as ascorbic acid, glutathione, cysteine and thiouracil[3,4,7] reduce DOPA-quinone to DOPA and thus block the further oxidation to pink halochrome. In the absence of a reducing agent, the DOPA-quinone is oxidized and undergoes an internal rearrangement to a colorless leukobody. This is then oxidized to a pink quinone, halochrome. Halochrome undergoes an internal rearrangement to a colorless compound and is further oxidized to melanin. Tyrosinase is necessary for the formation of DOPA and hence halochrome but not for the formation of melanin.[8]

Methods. *Preparation of Crude Tyrosinase.* Two types of potatoes, "new" and "Idaho Russet" potatoes, were used. No difference between their action was observed. They were washed thoroughly in tap water, distilled water and then peeled. The outer part of the potatoes was grated and the mash was squeezed through cheese-cloth. The fluid was centrifuged at 3000 to 3500 r.p.m. for 10 minutes. The supernatant was mixed with celite and then filtered through No. 5 Whatman paper. These procedures were performed in a cold room kept at 4° C. The use of celite as a filter aid was not part of the Duboff-Hirshfeld method. However, control experiments using tyrosinase prepared with and without celite showed no essential difference in their reaction with 3 normal and 3 cancer sera.

Collection of the Serum. Blood was drawn 2 to 3 hours before the experiment. The blood was kept at room temperature for 15 to 30 minutes. The clot was rimmed and

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the tube centrifuged at 3000 r.p.m. for 10 minutes. The serum was pipetted off into a clean tube and kept at 4° C. until used.

Solutions. M/15 sodium phosphate buffer of pH 7 to 7.05 was prepared; 0.001 M l-tyrosine in M/15 phosphate solution was freshly prepared every 4 to 5 days and kept at 4° C. when not used.

Preparation of Potato Tyrosinase for Standard Test. Five ml. of 0.001 M l-tyrosine, 0.5 ml. of phosphate buffer and 0.5 ml. of potato juice filtrate were mixed in a Klett-Summerson tube and stirred with a footed stirrer. A reading was made immediately in a Klett-Summerson photoelectric colorimeter (green filter No. 54). Fifteen minutes later, another reading was made. The potato juice was then diluted with buffer to the tyrosinase activity recommended by Duboff and Hirshfeld *viz.* one which produces a color development giving a change of about 0.6 Klett scale units per minute.

Determination of the Activity of Tyrosinase-tyrosine System in the Presence of Serum. Three pairs of Klett colorimeter tubes were set up as follows:

Pair No.		
	Constant Components	
1	5.0 ml. 0.001 M l-tyrosine 0.5 ml. standard potato juice	
2	Same as 1	
3	Same as 1	
	Variable Components	
1	0.5 ml. phosphate buffer	
2	0.5 ml. normal serum	
3	0.5 ml. cancerous serum	

The tubes were examined *seriatim* as follows: The serum or phosphate buffer was added to the tyrosine and was immediately followed by the standardized potato juice. The mixture was stirred and read (usually within 0.5 minute) in the colorimeter. Readings were made every 2 minutes for 5 readings and then every 5 minutes for a total of 30 minutes, the times being noted to the nearest 10 seconds.

In the early part of the investigation, the solutions were mixed in the dark and immediately placed in an illuminated bath for color development in accordance with the original description of Duboff and Hirshfeld. Later this operation was discontinued as unnecessary, an experience in accord with that of Duboff and Hirshfeld (personal communication).

Calculation of Tyrosinase Activity. The changes of the scale readings of the colorimeter were plotted against time. The resultant curve usually showed characteristics illustrated in Figure 1, *viz.*, (1) a *lag phase* during the initial 8 to 12 minutes. This is characterized by either no change in reading or a decrease indicating a diminution of the color, which was always initially present in the reaction mixture. (2) An *activity phase* characterized by a rectilinear change in colorimetric readings indicating constant rate of color production.

On the basis of these curves, the inhibition of tyrosinase activity was calculated by 3 methods: (1) That of Duboff and Hirshfeld. The time at which the *control* tube showed an increase of color of 12 Klett scale units was noted. The increase of reading (R) in the *serum containing tubes* at this time was also recorded. The percentage inhibition was calculated as follows: $100 \times \frac{R - 12}{12}$. By this convention inhibition is negative in sign. (2) The second datum was the *lag phase*, *i. e.*, time between the initial reading and the *beginning* of the constant increase in color formation. As a rule this could be estimated within 1 minute. (3) The *activity phase* was quantitated by calculating the activity of color development from the slope of the line in colorimetric scale units per minute. The result was expressed in percentage increase or decrease from the rate of the control tube without serum, *viz.*,

$$\frac{\text{Activity in the serum tube}}{\text{Activity in the control tube}} \times 100$$

Results. The sera of 24 cases of neoplastic disease and of 24 non-cancerous controls were examined. The diagnosis of neoplastic disease was established beyond

reasonable doubt in each case by either biopsy, operative report, or autopsy in addition to the clinical findings. The non-cancerous cases were patients suffering from miscellaneous ailments which had been definitely diagnosed as non-cancerous. In addition a number of normal subjects were tested.

The data are given in Table 1. In most cases duplicate determinations were done and the mean values were used for the purposes of the summary. Statistical calculation of the means ± the standard error of the means for both series shows that there is no significant difference between the two.

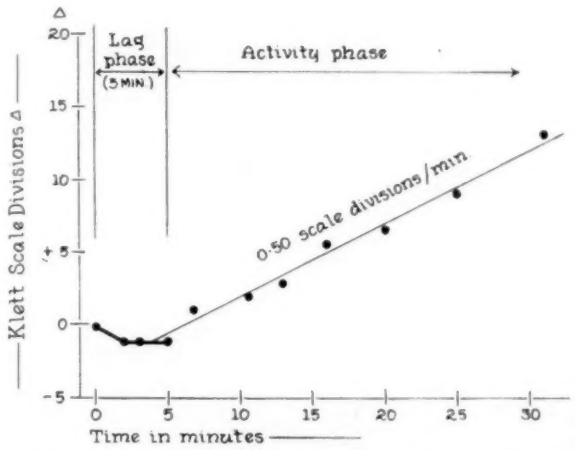

FIG. 1.—Typical curve of color development in a tyrosinase-tyrosine serum system showing (*a*) lag phase and (*b*) activity phase.

Method 1, that advocated by Duboff and Hirshfeld for the measurement of tyrosinase activity, suffers from a serious technical difficulty. During the initial 2 to 4 minutes of the lag phase, rapid decrease of the intensity of color, as measured by the Klett colorimeter, was frequently observed. This abrupt change of the color intensity was noted in the tubes containing buffer as well as those with sera. Hence this decrease in color could not be attributed solely to a constituent of the sera. Because of the rapidly changing color intensity, the initial reading was not considered reliable to within ±2 Klett scale units. In the determination of the inhibition, this error in the initial reading is equivalent to an error of ±16%. This conclusion is borne out by a calculation from the data of the entire series of the standard error of the mean of duplicate determinations on a single serum. For Method 1 (see table) it is about 15% and for Method 3 it is 7%. The latter method is somewhat more reliable, but both would require differences of 20 to 45% of inhibition between 2 sera to exist before any significance could be attached to the difference.

Another series of 13 cancer and 18 non-cancerous sera were examined using 2 modifications: (1) The initial reading was taken 10 minutes after the components of the system had been mixed. (2) Potato juice of varying tyrosinase activity was used. The inhibition exceeded 15% in 5 of the 13 cancer sera and in 5 of the 18 non-cancerous sera. Method 3 was used in these calculations. The results are presented in Table 2. Variations in the strength of the potato juice did not significantly alter the inhibition. In this series, as in the first series, there is no significant difference between the cancerous and the non-cancerous sera with

respect to tyrosinase inhibition, as shown by inspection and statistical analysis.

Discussion. The application of simple statistical methods to the evaluation of the data in both of these series of cases shows that there is no relation between the inhibition by serum of the tyrosinase activity as measured by any of the 3 methods outlined and the cancerous state. We have no explanation for the striking

TABLE 1.—TYROSINE INHIBITION BY CANCEROUS AND NON-CANCEROUS SERA

Type of case	Method 1 (%)	Method 2 (min.)	Method 3 (%)
Cancer:			
Tongue	−16, −42	2, 4	−42, +17
	−33, −58	0, 8	−22, −32
Lip	+12, −12	8, 6	−14, −10
Colon	+ 8, − 4	4, 4	+11, 0
Rectum	+ 8	0	− 7
	−42, + 8	4, 3	−10, + 8
Uterus	− 8, + 8	2, 4	−10, − 8
Cervix	−17, − 8	2, 2	0, −12
	−16, − 4	6, 8	− 3, −17
Bladder	−12, + 8	4, 0	− 4, 0
	+20, − 8	0, 6	+40, +33
	−35, −30	3, 3	−15, −13
Urethra	−50, −54	8, 6	+11, −10
Prostate	−25	8	−11
Kidney	0, 0	9, 9	−13, −18
Lung	0, −13	3, 4	+ 5, 0
	− 8, + 8	0, 5	−24, −19
	−17, −25	5, 10	−25, −14
	− 8, −25	2, 2	−24, −27
Breast	+ 8, −33	1, 2	− 4, − 4
	+ 4, − 4	2, 4	0, + 8
	− 8, + 8	0, 2	−11, −18
Leukemia:			
Myeloid	−25, −25	4, 3	− 7, 0
Lymphoid	0, −33	0, 2	− 5, − 2
Mean	−13 4 ± 3.3 (S.E.M.)	4.2 ± 0.6 (S.E.M.)	−7 1 ± 2.7 (S.E.M.)
Non-cancer:			
Normal	−70, −50	4, 2	−26, −20
Cyst of neck	−46, −33	3, 2	−17, − 8
Arthritis	0, − 4	2, 4	+ 3, 0
Syphilis	+16, + 8	2, 2	−17, −12
Thrombocytopenia	−42, 0	7, 6	−15, − 3
Coronary thrombosis	+17, +25	4, 3	0, −13
Arteriosclerosis	+ 8, +25	10, 4	0, 0
Appendicitis	−25, +33	0, 0	− 7, + 6
Ulcerative colitis	−25	0	−33
Hepatitis	−37, −16	3, 4	− 7, −12
Cholecystitis	−12, −29	4, 5	−12, −11
Cholelythiasis	−25, −46	5, 4	−16, −33
Urethral stricture	−29	0	+18
Prostatic hypert.	−35, −38	0, 15	−36, −20
Tbc. of scrotum	+ 4, 0	0, 0	+10, − 8
Tbc. of bone	−46, +21	0, 6	−14, −14
Hernia	−75, −46	3, 5	−43, −44
	−38, −46	7, 12	−17, − 8
Cystocele	−58, −12	2, 0	−10, −33
	−16, +12	2, 0	−16, −14
Cervicitis	− 8, − 8	4, 2	−10, − 8
	− 4, −33	0, 4	−17, 0
	−29, − 8	2, 2	−18, −16
Fracture	+75, +42	0, 0	+44, +20
Mean	−16.3 ± 5.4 (S.E.M.)	3.0 ± 0.5 (S.E.M.)	−11.0 ± 3.2 (S.E.M.)

Standard error, duplicate determinations: Method 1, 14%; Method 3, 7%.

disparity between our results and those of Duboff and Hirshfeld.

However, it was found that some sera did alter the tyrosinase activity of the system in 2 respects: (1) an increase, though very variable, in the time of the *lag phase*, and (2) a decrease, sometimes large, of the rate of color production during the *active phase*. In an attempt to obtain information about these aspects some experiments were done, initiated by the observations of Nelson and Dawson.[6] They reported that in a tyrosinase-tyrosine system containing ascorbic acid the DOPA quinone formed was reduced as soon as formed by the ascorbic acid. Hence there was no color development. When the ascorbic acid was all oxidized, color development promptly began and continued at a rate comparable to controls to which no ascorbic acid has been added. Gluthathione[4] has also been reported to inhibit tyrosinase activity in a similar fashion.

This phenomenon is paralleled by our observations with serum and it is conceivable that the variations in the lag phase could be explained by assuming variable concentrations of reducing substances in the sera. We observed that the addition of 5 uM of ascorbic acid or 1 uM of glutathione decolorizes the customary pink initial color of the potato tyrosinase and in addition prevented new color formation for over an hour. With 0.5 uM of ascorbic acid or 0.05 uM of glutathione a more limited cessation of color formation was observed. After a delay period of about 20 minutes, the color formation started abruptly and its rate usually *exceeded* that of the control. It was observed, however, that 0.1 uM of ascorbic acid had no influence on the tyrosinase-tyrosine system. This is the amount roughly equal to the average amount of ascorbic acid in the 0.5 ml. of the human serum used for the test. Hence the effective amount of ascorbic acid needed to inhibit the system is greater than the usual blood concentration. Confirmation of this conclusion was obtained in the following experiment. The sera of 2 normal individuals were tested before and after the ingestion of 1 gm. of ascorbic acid. No significant differences were observed in either the lag or activity phase of the preprandial or postprandial sera when tested with the tyrosinase system.

However, 0.05 uM of glutathione added to the assay system did cause a significant increase in the delay phase. The amount

TABLE 2.—TYROSINASE INHIBITION BY CANCEROUS AND NON-CANCEROUS SERA (METHOD 3)

Type of case	% change from control rate (activity phase)
Cancer of:	
Face	− 7
Tongue	−18
Palate	−12
Stomach	−20
	−15
Liver	−10
Rectum	−22
Bladder	0
Prostate	− 8
Lung	−16
	−12
Mediastinal sarcoma	−13
Hodgkin's disease	−14
Mean	−12.8 ± 1.6 (S.E.M.)

Type of case	% change from control rate (activity phase)
Normal	−11
	−11
	− 7
	− 7
	−14
	−11
	−19
	−24
	−12
	−13
Simple fracture	− 8
Infected finger	−12
Urethral stricture	−20
Hemorrhoids	− 3
Glaucoma	−10
Coronary thrombosis	−18
Non-toxic goiter	− 2
Fibromyoma uteri	−27
Mean	−12.7 ± 1.6 (S.E.M.)

is roughly one-third of that usually found in 0.5 ml. of normal serum.

These experiments suggest the possibility that variations of reducing substances, such as glutathione, might be responsible for the increase in the *delay phase*. But the variations in the *activity phase* remained unexplained, for neither ascorbic acid nor glutathione produced any *decrease*, but rather an increase in this activity of the tyrosine-tyrosinase system.

Summary. 1. The suggested serodiagnostic test for malignancy based upon the tyrosinase inhibiting action of serum from cancerous patients, reported by Duboff and Hirshfeld, was studied.

2. The characteristic effect of serum upon the development of color of a l-tyrosine, potato-tyrosinase system could be divided into 2 parts, *viz.*, a *lag phase* and an *activity phase*. On this basis the effect of serum could be expressed in 3 ways, 1 of which is identical with that used by Duboff and Hirshfeld.

3. No significant differences were observed between the effects of sera from 37 cancerous and 42 non-cancerous subjects on the tyrosinase system, and we conclude that the test has no serodiagnostic value in malignancy. The disparity between our results and those of Duboff and Hirshfeld remain unexplained.

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THE TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM WITH PENICILLIN*

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This report presents the clinical results, the serologic data and the changes in the spinal fluid in 141 patients with neurosyphilis treated with penicillin. These patients have been followed from 6 to 19 months. The principal factors which are known to influence the treatment of neurosyphilis, such as the duration and severity of the disease, the degree of spinal fluid abnormality, and the dosage of penicillin, are analyzed.

Material and Procedures. All of the 141 patients in this series have been treated and observed in this clinic. Of these, 83 were male and 58 female; 51 were white and 90 were Negro. The diagnoses of these cases at the onset of treatment were:

Acute meningitis	4
Early asymptomatic neurosyphilis	19
Late asymptomatic neurosyphilis	74
Congenital asymptomatic neurosyphilis	5
Meningovascular syphilis	26
Tabes dorsalis	4
Paresis	7
Optic atrophy	2

The group of patients diagnosed as having meningovascular neurosyphilis consisted chiefly of those with pupillary abnormalities, hemiplegias or cranial nerve lesions. Four of the patients in this group had other manifestations of syphilis, such as transverse myelitis, eighth nerve deafness, and meningomyelitis.

Before beginning treatment, a complete history was taken on each patient and a physical examination, including a neurologic and mental status evaluation, was done. Quantitative serologic tests and spinal fluid examinations were also made at this time. Cell counts on the spinal fluid were done within an hour after the spinal fluid was taken. Quantitative spinal fluid protein determinations were made by electrophotometric measurement of the turbidity produced on the addition of 4 cc. of 3% sulfosalicylic acid to 1 cc. of spinal fluid. The turbidity reading was then compared with that of a known standard protein solution and the level of the spinal fluid protein was determined. Micro-Kjeldahl determinations of the spinal fluid protein were done as a check on the electrophotometer method in several patients in whom the protein level was elevated one year after treatment. In each of these cases the elevated protein determination of the electrophotometer was confirmed. The Kolmer complement fixation test was done with serial dilutions of spinal fluid from .0312 to .5 cc. Colloidal tests were done with mastic solution. The quantitative Kahn test was used on all blood specimens.

At the onset of treatment, the spinal fluid in 81 of the 141 patients in this series showed a definite increase in both cells and protein, in addition to a positive Wassermann reaction. In these 81 cases, the cell count was greater than 10 per cu. mm., the protein more than 45 mgm. %, and the Kolmer was usually positive with less than .25 cc. of spinal fluid. This type of fluid conforms with the Group 3 classification of Moore.[4] The spinal fluids of 54 patients showed an elevation of *either* cells *or* protein, in addition to a positive Wassermann, and these were classified as Group 2. The remaining 6 patients had inactive Group 2 fluids with a positive

* The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for clinical investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

We are indebted to Miss Jeanne Pettay, for performing the spinal fluid tests.

Wassermann, but with normal cells, protein and mastic.

At the beginning of this study, a total dose of 1.2 million units of penicillin was used in each patient. This dose was later increased to 2 to 3 million units. Sodium penicillin was dissolved in isotonic salt solution and given in equally divided doses intramuscularly every 3 hours. At present, we are using a total dose of 4 million units of penicillin in doses of 50,000 units every 3 hours for 80 injections in 10 days. Cases of primary optic atrophy and general paresis are now receiving a total dose of 6 million units in 18 days. Penicillin is administered in these patients in gradually increasing amounts for the first 3 days to minimize the intensity of the Herxheimer reaction, and then in doses of 50,000 units every 3 hours for the following 15 days. In this paper only the results of one course of penicillin therapy are considered. Each patient was instructed to return at three-month intervals for further physical examinations and for serologic and spinal fluid studies.

Seventy-seven patients were followed for 6 to 8 months, 35 patients from 9 to 11 months, while the remaining 29 patients were followed for 12 to 19 months.

Results. Changes in Spinal Fluid Following Treatment. The response of the spinal fluid to penicillin therapy has been classified as follows:

1. *Return to Normal.* This indicates that a complete reversal of the spinal fluid has occurred with the cell count below 10 per cu. mm., the protein level below 45 mgm. %, the mastic reading less than 111000, and the Kolmer reaction negative with .5 cc. of spinal fluid.

2. *Satisfactory Response.* This group includes those spinal fluids in which the cells, protein and mastic have become normal, but the Wassermann reaction remains positive, usually with a decrease in titer. This series of changes probably represents an inactive neurosyphilitic process and we regard it as a satisfactory response to treatment. (Dattner[2]).

3. *Improved.* This group of cases includes those spinal fluids in which the cell count has become normal, but the protein level remains above 45 mgm. %. The mastic in this group of cases is often greater than 111000, and the Wassermann reaction is always positive, although usually decreased in titer. The protein in most of the patients in this group had fallen from a much higher level, but in a few instances it had remained stationary.

4. *Evidence of Relapse.* Examination of the spinal fluid in this group of patients showed a significant elevation of the cell count (usually 50 cells or more per cu. mm.) 6 months after treatment, indicating that an active neurosyphilitic process existed and that treatment had failed. These patients usually had a normal or decreased cell count 3 months following treatment, but there was a significant rise in cells at the end of 6 months. In an occasional patient, the spinal fluid contained 10 to 20 cells per cu. mm. 6 months following therapy. This was not regarded as evidence of relapse if the cell count had been steadily decreasing. If, however, the cell count remained above 10 per cu. mm. 1 year after treatment was completed, the patient was regarded as a treatment failure and was re-treated.

Changes in Spinal Fluid in Relation to Dosage of Penicillin. Table 1 shows the effect of penicillin therapy on the spinal fluid findings in relation to total dose of penicillin received. In this study, the use of 4 million units of penicillin was the most recently employed dosage schedule. Consequently, the period of follow-up observation in these patients is not as long as in those receiving smaller doses. This factor is of great importance and must be considered in comparing the effects of the various doses of penicillin.

It will be observed that 4 of the 10 patients who received doses of 1.2 million units of penicillin developed evidence of relapse. One patient obtained a normal spinal fluid, while the remaining 5 cases had a satisfactory response. The time the relapses appeared is classified in Table 2

according to the dosage of penicillin and the number of patients under observation. Three of the 4 patients who relapsed following 1.2 million units of penicillin had a significant pleocytosis in the spinal fluid 6 months following treatment, while the remaining patient had a slightly elevated cell count at the end of 1 year.

Table 1.—Changes in Spinal Fluid in Relation to Dosage of Penicillin

Dose of Penicillin in Million Units*	Number of Patients	Spinal Fluid Response			
		Relapsed	Normal	Satisfactory	Improved
1.2	10	4	1	5	..
2.0 to 3.0	63†	7	6	32	18
4.0	63	2	9	34	18

* Three patients with paresis treated with 6 million units are not included. Likewise, 2 children with late congenital neurosyphilis treated with 40,000 units per kilogram of body weight are omitted.

† Of these patients 45 were treated with 2.4 million units.

Table 2.—Relation of the Time of Occurrence of Spinal Fluid Relapse to Dosage of Penicillin and Number of Cases Observed

Dosage of Penicillin (million units)		Months After Treatment		
		6–8	9–11	12–19
1.2	No. patients under observation	10	7	6
	No. patients relapsing at specified time	3	..	1
2.0–3.0	No. patients under observation	63	41	22
	No. patients relapsing at specified time	4	1	2
4.0	No. patients under observation	63	15	..
	No. patients relapsing at specified time	2	..	..

Sixty-three patients received between 2 and 3 million units of penicillin. Of these 7 relapsed, 6 obtained normal spinal fluids, while the remaining improved or had a satisfactory response (Table 1). Of the 7 patients who developed a relapse, 1 had received 2.0 million, 3 had received 3.0 million, and 3 others, 2.4 million units of penicillin. Four of the 7 relapses appeared 6 months after treatment, 1 at 9 months, while the remaining 2 cases were regarded as relapses only at the end of 1 year (Table 2).

Of the 63 patients receiving 4 million units, 2 relapsed, 9 obtained normal spinal fluids, while the remaining cases obtained satisfactory results or improved. Both of the relapses in this group occurred 6 months following treatment.

The patients treated with the various doses of penicillin were in general comparable as to the type of neurosyphilis. There were, however, more patients with early neurosyphillis treated with 4 million units of penicillin than with smaller doses. This would account to some extent for the large number of normal spinal fluids obtained with this dose.

Although more relapses occurred in patients receiving smaller doses of penicillin, the differences in the period of follow-up observation must be considered. None of the patients receiving 4 million units of penicillin were followed for as long as a year, and relatively few of this group have been observed longer than 9 months. All of the patients in this study, however, have been observed for at least 6 months following treatment and a comparison of the relapses occurring at this period of time is shown in Table 2. Although the number of patients treated with 1.2 million units of penicillin is small, the appearance of spinal fluid relapses six months after treatment in almost one-third of these cases indicates that this dosage is inadequate. In the two groups of patients treated with 2 to 4 million units, the number of relapses occurring 6 months after treatment was not significantly different. It is our belief, however, that doses of penicillin less than 4 million units will prove to be unsatisfactory in the treatment of neurosyphilis and should not be used in this condition.

Relation of Spinal Fluid Changes to Duration of Disease. Table 3 shows the changes in the spinal fluid classified according to the duration of the disease. It will be noted that approxi-

mately one-half of the patients known to have early neurosyphilis (less than 5 years' duration) developed a normal spinal fluid, whereas only 6 of the 118 patients who appeared to have late syphilis obtained a reversal of the spinal fluid. Likewise, there were many more spinal fluid relapses in the late cases of neurosyphilis.

of 211000, and a positive Wassermann with .0625 cc. of spinal fluid. This patient had no clinical evidence of neurosyphilis. He was given 4 million units of penicillin and 6 months later his spinal fluid showed 165 cells, 54 mgm. % of protein, with no change in the Wassermann or mastic reactions. His lack of response to penicillin is interesting in view of his failure to

TABLE 3.—RELATION OF SPINAL FLUID CHANGES TO DURATION OF DISEASE

Duration of Infection	Number of Patients	Spinal Fluid Response			
		Relapsed	Normal	Satisfactory	Improved
Early neurosyphilis	23*	1	10	12	..
Late neurosyphilis†	118	12	6	62	38

* Eighteen of these patients were treated with 4 million units of penicillin.

† These cases included those of unknown duration, presumed to be more than five years.

TABLE 4.—RELATION OF SPINAL FLUID CHANGE TO FINDINGS IN THE FLUID PRIOR TO TREATMENT

Findings in Spinal Fluid Before Treatment	Number of Patients	Spinal Fluid Response			
		Relapsed*	Normal	Satisfactory	Improved
Inactive Group II	6	..	1	5	..
Group II	54	4	9	33	8
Group III	81	5†	6	36	30

* Six months after treatment.

† Four additional patients with Group 3 spinal fluid findings showed evidence of relapse later than 6 months following treatment.

None of the patients with early neurosyphilis had initial primary or secondary syphilitic manifestations; the duration of the infection in these cases was usually 2 to 3 years. Several of them had relapsing cutaneous manifestations, but most of them had an asymptomatic neurorecurrence or meningitis.

The good results obtained with the use of penicillin in the treatment of early neurosyphilis has also been noted by others. Nelson[5] reported clinical cure and spinal fluid reversals in a number of patients with acute meningitis treated with penicillin, while O'Leary[6] states that penicillin therapy was most promising in the treatment of the meningeal form of this condition.

The 1 patient in this series with early neurosyphilis who developed a spinal fluid relapse was a 21 year old white man who had a penile lesion 3 years previous to his penicillin therapy. Despite almost 3 years of continuous and regular arsenical and bismuth treatments at various prison camps, this patient's spinal fluid showed 98 cells, 30 mgm. % of protein, a mastic

respond to adequate treatment with arsenic and bismuth.

RELATION OF SPINAL FLUID FINDINGS BEFORE TREATMENT TO THE CHANGES FOLLOWING PENICILLIN The response of the spinal fluid to penicillin therapy is classified in Table 4 according to the findings in the fluid immediately prior to treatment.

Most of the patients treated early in this study had Group 3 spinal fluids. This group of patients therefore have the longest period of follow-up observation and have also received the smaller doses of penicillin. These two factors must be considered in comparing the results obtained with Group 2 and Group 3 spinal fluids.

Approximately the same number of relapses occurred six months after treatment in patients with Group 2 and Group 3 fluids. There were, however, more patients in Group 2 developing normal spinal fluids than in Group 3. Although these findings may be the result of differences in dosage, it is nevertheless our impression that patients having maximal

spinal fluid changes obtained less satisfactory results following penicillin therapy.

Only one of the patients in this series with inactive Group 2 spinal fluids (those with normal cells, protein and mastic, but with a positive Wassermann) obtained any significant change in his spinal fluid findings, and this was a case of early neurosyphilis. In late cases of syphilis, this type of spinal fluid represents an inactive neurosyphilitic process (Dattner[2]). We think it probable that little or no change in the spinal fluid can be expected in these cases following penicillin therapy.

There were 38 patients in this entire series who were classified as improved and showed normal cell counts following treatment, but had persistently elevated spinal fluid proteins and positive Wassermann reactions. These cases are of considerable interest. Many of these patients have had a significant decrease in the spinal fluid protein and their present level is only slightly higher than 45 mgm. %. Since the elevation of the protein is usually much slower to subside than the pleocytosis, these patients cannot be called treatment failures at this time.

There were, however, 12 patients who had an increase in spinal fluid protein at the onset of treatment who still show an appreciable elevation in protein (more than 60 mgm. %) despite penicillin therapy. Three of these patients had normal cell counts before treatment. Significantly, all of these cases had syphilis of long duration, and one-half of them had either meningovascular syphilis or had definite parenchymatous involvement, such as paresis or tabes. Elevation of the spinal fluid protein in such cases has been found to persist for as long as 4 years or more following fever therapy.[2,3] It has been observed, moreover, that paretics with very high spinal fluid proteins show considerable tendency to maintain such levels almost continuously following fever therapy and that the clinical results in these patients are no different from those with low spinal fluid proteins.[3] In meningitis and in early neurosyphilis, however, we have observed a rapid and dramatic fall of extremely high protein levels following penicillin or arsenicals. The persistence of a high protein in the spinal fluid in our patients may therefore represent a severe degree of destruction in the central nervous system and does not necessarily indicate that penicillin was ineffective. It is this group of cases, however, that needs most prolonged and careful observation before the value of penicillin can be accurately assessed. Some of them probably will become treatment failures.

Relation of Spinal Fluid Response to Blood Serologic Tests. No apparent relation was noted between the results obtained in the spinal fluid and those obtained in blood serologic tests (Table 5).

None of the patients who developed a relapse in spinal fluid showed a rise in the titer of the quantitative Kahn. The Kahn titer fell in 7 of these cases, but remained the same in 5 others. None of these patients developed a negative blood serologic test.

Of the 16 patients who developed a normal spinal fluid, only 8 obtained a negative serologic reaction. The majority of the patients in this study showed a decrease in the titer of the blood serologic test regardless of the spinal fluid response. In many of the patients, however, no change in blood titer was noted, despite a satisfactory response or improvement in the spinal fluid.

The lack of correlation between blood and spinal fluid findings following penicillin therapy is noteworthy. It is easily understood how patients with relapsing neurosyphilis can be overlooked if one relies entirely upon the blood serologic test. In this study the majority of patients with evidence of neurorelapse showed no clinical symptoms, and in such cases, failure to respond to treatment can only be diagnosed early by repeated examinations of the spinal fluid.

Relation of Spinal Fluid Response to Type of Neurosyphilis. As suggested in the foregoing paragraphs, the types of

neurosyphilis that seemed to respond best to penicillin therapy were those with early asymptomatic neurosyphilis or acute syphilitic meningitis. This group of cases obtained the greatest number of normal spinal fluids and developed the smallest number of relapses (Table 6).

All but 1 of the 5 patients with late congenital asymptomatic neurosyphilis obtained a satisfactory spinal fluid response. This patient had 200 mgm. % of protein in the spinal fluid and no cells prior to treatment, and 11 months later showed only a slight reduction in the spinal fluid protein.

The large number of patients with parenchymal neurosyphilis who maintain an elevation of the spinal fluid protein has been mentioned previously in this report. Since this group of patients have most destruction of the central nervous system, and since the ultimate response of such spinal fluids to penicillin is not known, the importance of prolonged observation of this particular group of patients is again emphasized.

THE RELATION OF SPINAL FLUID RESPONSE TO PREVIOUS TREATMENT.

TABLE 5.—RELATION OF SPINAL FLUID RESPONSE TO BLOOD SEROLOGIC TESTS

Results of Serologic Tests of Blood	Number of Patients	Spinal Fluid Response			
		Relapsed	Normal	Satisfactory	Improved
Became negative	34*	..	8	18	8
Decreased in titer	58	7	7	33	11
No change	46	5	1	21	19
Increased in titer	..	..	..	..	..

* Of these patients 12 had negative or doubtful blood serologic tests at onset of therapy.

TABLE 6.—RELATION OF SPINAL FLUID RESPONSE TO TYPE OF NEUROSYPHILIS

Diagnosis at Onset of Therapy	Number of Patients	Spinal Fluid Response			
		Relapsed	Normal*	Satisfactory	Improved
Acute meningitis	4	..	2	2	..
Early asymptomatic	19	1	8	10	..
Congenital asymptomatic	5	..	..	4	1
Late asymptomatic	74	10	6	41	17
Meningovascular	26	2	..	15	9
Tabes	4	..	..	..	4
Optic atrophy	2	..	..	2	..
Paresis	7	..	..	..	7

* Of these patients 5 have a doubtful or a 2-plus Kolmer reaction with 0.5 cc. of spinal fluid.

Seventy-four patients in this series appeared to have late asymptomatic neurosyphilis. Most of these responded well to treatment and obtained satisfactory or normal spinal fluids. Ten of them relapsed, however, and 17 others maintained elevated spinal fluid proteins.

None of the patients with meningovascular neurosyphilis, paresis, tabes or optic atrophy obtained normal spinal fluids. Less than one-half of them showed a satisfactory response, and many of them showed persistently elevated proteins. These findings are in general in accordance with those noted by others[7] and demonstrate that the spinal fluid response to penicillin is poorest in the more advanced cases of neurosyphilis.

The therapy our patients received prior to penicillin treatment has been classified into 4 groups: (1) No treatment; (2) poor chemotherapy, consisting of less than 25 arsenic and 25 bismuth injections; (3) adequate chemotherapy, consisting of more than 25 arsenic and 25 bismuth injections, regardless of the regularity and time over which it was given; (4) fever therapy.

There did not appear to be any correlation between the failure of the spinal fluid to respond to penicillin and the amount of previous treatment. Of the 13 cases who developed evidence of relapse, 3 had had no previous therapy;

2, poor therapy; 7, adequate therapy, and 1 had had fever.

The effects of previous therapy, however, cannot be accurately evaluated. Greater amounts of treatment, including fever, were usually given to the more advanced cases, and the results of penicillin in these instances were less satisfactory.

It should be emphasized, however, that approximately one-half of the patients who obtained normal or satisfactory spinal fluid findings had had adequate chemotherapy previously, indicating that penicillin has a far greater effect on syphilis of the central nervous system than routine chemotherapy. This observation is, we believe, of considerable importance and indicates that penicillin should replace entirely the use of routine arsenic and bismuth preparations in the treatment of neurosyphilis.

Table 7.—Comparison of the Response of Spinal Fluid to Penicillin With That Obtained After Fever Therapy (Typhoid Vaccine)

Method of Therapy	Number of Patients	Spinal Fluid Response			
		Relapsed	Normal	Satisfactory	Improved
Penicillin, 4.0 million units	48	1	1	27	19
Fever therapy*	48	3	2	32	11

* Two of these patients received malaria.

Comparison of Response of Spinal Fluid to Penicillin Therapy With That Obtained With Fever. During the two years prior to the use of penicillin in this clinic, we had employed fever therapy routinely in the treatment of the same types of neurosyphilis we are now treating with penicillin. The spinal fluid responses of these two methods of therapy are compared in Table 7. Patients with early neurosyphilis are not considered, since fever was rarely used in these cases. Only patients who received at least 4 million units of penicillin are included in this comparison.

Observations of the spinal fluid response in patients treated with fever were usually made 12 to 18 months following therapy. The two groups of cases are otherwise comparable, particularly in regard to diagnosis and initial spinal fluid abnormalities. The course of fever therapy in this clinic consists of fifty hours of temperature greater than 103° F., obtained by intravenous infusion of typhoid vaccine in seven to ten courses. In 31 of the 48 patients considered in the table, no further treatment was given following fever therapy. In 17 cases, however, 10 to 20 weekly injections of tryparsamide were given after fever was completed. Since no significant differences could be observed in this small series in the spinal fluid response of those receiving fever alone and those receiving fever and tryparsamide, both groups are considered together for comparison with penicillin-treated patients.

In general, the number of patients obtaining a normal or satisfactory spinal fluid is approximately the same with the two methods of treatment. The number of relapses following fever was not significantly higher than that obtained with penicillin. It will be noted, however, that more patients maintained an elevated protein after penicillin than after fever, possibly because the period of follow-up observation was longer in patients treated with fever.

Although the response of the spinal fluid to penicillin appears to be equal to that obtained with fever, it must be remembered that clinical improvement does not always parallel the spinal fluid findings. It is extremely difficult to compare any two groups of patients with neurosyphilis in regard to the improvement in clinical symptoms, but it is our impression that the results of fever therapy were somewhat better than penicillin therapy in our patients with paresis. In asymptomatic neurosyphilis, however, or in patients in whom only an arrest in progress

may be expected, penicillin definitely appears to be the treatment of choice.

Response of Clinical Symptoms to Penicillin Therapy. The majority of patients in this series were asymptomatic at the onset of treatment or had irreversible meningovascular manifestations, such as fixed pupils and long-standing hemiplegias. It was not possible, therefore, to ascertain any objective clinical improvement following treatment in most of our patients.

There was, however, a surprising number of patients in this series who obtained marked subjective improvement. These patients frequently stated that they felt better, had gained weight, had more pep and vigor and were "one hundred percent different." Many of them also obtained relief from headaches, malaise and muscular aching, although these symptoms were not mentioned by them at the time of their initial examination. Such "tonic" effects have also been noted by others. O'Leary,[6] for example, found that weight gain was a common occurrence and noted that an over-all general improvement was frequently obtained.

The response of specific symptoms of neurosyphilis to penicillin therapy was, however, considerably more variable. In 2 patients with tabes, ataxia was greatly improved and the patients returned to work. On the other hand, 2 other patients with lightning pains obtained only temporary relief.

Six of the 7 patients with early paresis showed definite improvement and are now well enough to return to their former jobs. The response to penicillin was often very dramatic, with immediate improvement of tremors, handwriting and slurring of speech. One patient, however, showed only slight improvement in mental status. Records have been obtained from 6 other patients with paresis, who are not included in this series, as they were transferred to psychiatric institutions and could not be observed in this clinic. Four died and two remain psychotic. In all of these cases, however, the disease was far advanced at the onset of treatment.

Both patients with optic atrophy have shown progressive loss of vision and constriction of visual fields, despite the fact that their spinal fluids have responded satisfactorily. The patients with transverse myelitis, meningomyelitis and eighth nerve deafness have not shown any definite clinical improvement.

Reactions. The most common reaction encountered in our patients was an initial febrile Herxheimer effect. This usually occurred on the first day of therapy, but was sometimes delayed until the second or third day in those patients who received gradually increasing doses of penicillin. Three of the 7 patients with paresis became considerably agitated at the onset of therapy and had to be restrained, but this effect was only temporary. One patient (not included in this series) who had been diagnosed as asymptomatic before treatment, developed an acute psychosis during penicillin therapy, necessitating discontinuance of therapy. Although such reactions have been previously reported,[1] they are relatively uncommon.

Discussion. Only a relatively small number of patients in this series obtained normal spinal fluids after penicillin. This we believe is due to the comparatively short period of observation which has elapsed following treatment. As pointed out by Stokes and his associates,[7] there is a striking increase in the number of normal spinal fluids with longer periods of observation. We believe that most of the patients with a satisfactory spinal fluid response and some of those with improvement in the spinal fluid will soon obtain normal findings.

The criterion for relapse in this series has been based entirely upon the cell count, for this is believed to be the most sensitive indication of the activity of the neurosyphilitic process.[2] In 10 of the 13 patients in this series who relapsed, there appeared to be an initial improvement in the cell count, but approximately six months following therapy, the cells

rose again to the original level or higher. In the remaining 3 cases, the cell count showed a steady decline but was still greater than 10 cells per cu. mm. a year following therapy.

We have found the response of the protein to be considerably slower and not as sensitive as the cell count. The protein rose in only 3 of the 13 patients who showed an increase in cells. Changes in the mastic were generally reflected by the more accurate quantitative protein determinations. No change was noted in the mastic reaction in any of the cases of relapse.

Only marked changes in the Wassermann titer of at least 2 or 3 dilutions were regarded as significant in this study. Minor changes of 1 or 2 dilutions could not be depended upon as an indication of relapse because of the daily uncontrollable laboratory variation in the sensitivity of the complement fixation reaction. In this series, only 5 of the patients with relapse showed any significant changes in the Wassermann titer. As mentioned previously, the blood serologic test was of no value in the detection of relapsing spinal fluids.

Clinical symptoms were also of little importance as an early indication of treatment failure. Only 2 patients with relapsing spinal fluids developed any clinical signs. One of them noted a recurrence of headache, while the other suffered a transient monoplegia. For these reasons, we have come to depend almost entirely on the cell count for re-treatment of our asymptomatic cases, since neither the clinical, serologic, or other spinal fluid findings appeared sufficiently reliable for the early diagnosis of treatment failure.

In several patients there was a return or progression of symptoms, despite a satisfactory response or improvement in their spinal fluids. The decision to give further treatment to these patients depended upon the type and severity of their symptoms.

Patients who showed a relapse in the spinal fluid following small doses of penicillin were re-treated with 6 million units of penicillin. Those relapsing after large amounts of penicillin were given fever therapy, in addition to twice the original amount of penicillin.

In one of the patients in this series, the cell count was normal at 3 and 6 months, but increased 9 months following therapy. It appears therefore that, although most relapses occur within the first 6 months, patients treated with penicillin should be followed for considerably longer intervals. A somewhat greater relapse rate is to be expected in this series, therefore, with longer periods of observation. (See Addendum).

The total number of spinal fluid relapses, however, in this group of patients with neurosyphilis who received 4 million units of penicillin will be quite small and will probably be less than 15%. Penicillin is a very valuable agent in the treatment of this condition and should replace arsenic and bismuth preparations. Moreover, in asymptomatic neurosyphilis it is an effective and a safer method of treatment than fever. We believe that fever therapy is still the method of choice in most of the patients with late symptomatic neurosyphilis. The frequent and dramatic response of many of these cases to penicillin, however, and the safety of this form of therapy make the use of penicillin warranted in earlier and milder forms of this condition.

Conclusion. One hundred and forty-one patients with syphilis of the central nervous system were treated with doses of penicillin ranging from 1.2 to 6 million units and were followed for 6 to 19 months. Our results are in general similar to those reported by others and penicillin appears to be an effective agent in the treatment of this condition.

Preliminary observations on the use of 4 million units of penicillin indicate that this dosage will probably produce an arrest of the neurosyphilitic process in more than 85% of the cases.

Patients with early neurosyphilis obtain a better response to penicillin than late

cases. It is our impression that the results in patients with maximal spinal fluid findings in late syphilis are not as good as those with less severe spinal fluid findings. Moreover, patients with late symptomatic neurosyphilis seemed to obtain less satisfactory spinal fluid responses than those without symptoms. In general, however, the response of the spinal fluid in late cases of neurosyphilis was as good as that obtained with fever therapy.

No correlation was observed between the changes in the blood serologic tests and the response of spinal fluid following penicillin.

Marked subjective improvement and a sense of well-being were frequently noted in patients with neurosyphilis treated with penicillin. Although the clinical improvement in paresis and tabes was occasionally striking, it did not always parallel the improvement of the spinal fluid in these cases.

The cell count of the spinal fluid was found to be a sensitive index of treatment failure. In those patients developing a relapse after penicillin, a significant elevation in the cell count was usually noted six months after treatment. Occasionally, however, longer periods of observation were necessary before evidence of relapse could be detected. In this study, 13 of 141 patients were found to have evidence of spinal fluid relapse following penicillin therapy. There were, in addition, a small group of patients who developed a recurrence of clinical symptoms or a progression of their neurosyphilitic manifestations following therapy.

Penicillin is recommended for the treatment of early neurosyphilis and late asymptomatic neurosyphilis provided adequate facilities are available for follow-up examinations of the spinal fluid. In late symptomatic neurosyphilis penicillin does not replace fever therapy as the treatment of choice. The safety of penicillin therapy, however, and the frequent dramatic results obtained in these cases warrant the use of penicillin in earlier and milder forms of this condition.

The patients in this study have been followed for only a relatively short period of time, and these conclusions must therefore be regarded as preliminary. The results described in this paper, moreover, must be interpreted in light of the fact that commercial penicillin has been a changing mixture of various substances. The content of impurities has gradually decreased as potency in terms of units per milligram has increased. The relative amounts of the several identified penicillin fractions G, F, X and K, have likewise varied from time to time. These two changes and perhaps others suggest that therapeutic efficacy may not have remained constant, and that it may be significantly different today than it was originally. It is not now possible to assess the extent to which these changes may have affected the results here reported.

Addendum. Since this paper was submitted for publication, evidence of spinal fluid relapse has appeared in 3 of the patients with late neurosyphilis treated with 4 million units of penicillin. Each of these patients showed 17 to 20 cells per c.mm. a year after treatment was completed.

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THE ORAL ADMINISTRATION OF PENICILLIN IN DOGS

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This report describes the results of an investigation of the effectiveness of various substances for the protection of orally administered penicillin in dogs. The manner in which various substances protect penicillin against destruction by gastric acidity has been reviewed.[1] Experiments in humans with certain oral penicillin preparations have been reported by Finland *et al.*[2]

The protective substances for the animal experiments were selected on the basis of their ability to protect penicillin against acid inactivation as indicated by *in vitro* experiments with artificial gastric juice. The protective substances investigated included alkaline buffers, antacids, proteinic materials and oils. Commercial penicillin calcium was used throughout the study.

In Vitro Studies. Measurement of the pH values after the addition of successive portions of soluble, alkaline salts to 25 cc. portions of artificial gastric juice* indicated that certain of these salts were capable of buffering the juice at a pH range compatible with the stability of penicillin. The effect of trisodium citrate, sodium acetate and dibasic sodium phosphate is illustrated in Figure 1. Several other antacids were also found to adjust the pH of the juice to a satisfactory level. The effect of calcium carbonate and tricalcium phosphate are illustrated in Figure 2. It is apparent from Figures 1 and 2 that these substances maintained the pH in a satisfactory range even after the addition of more artificial gastric juice. Under these conditions penicillin was protected against rapid destruction.

The protective effect of certain buffers and antacids was demonstrated in the following experiments. In artificial gastric juice to which no buffers or antacids were added, 20,000 units of penicillin calcium was completely inactivated after incubation at 37.5° C. for 30 minutes. In samples of artificial gastric juice containing 20,000 units of penicillin calcium and 750 mg. of disodium phosphate, sodium acetate or trisodium citrate respectively, 80% of the drug was present after incubation at 37.5° C. for 30 minutes. A similar protective effect was observed when 500 mg. of calcium carbonate or tricalcium phosphate was used.

Several protein materials, including soluble egg albumen, skimmed milk powder and whey powder were ineffective in raising the pH of artificial gastric juice to a range compatible with penicillin stability. Nevertheless, under certain conditions, these substances protected penicillin against acid inactivation. A control solution buffered to pH 3, containing 100 units of penicillin per cc., lost 25% of its activity during incubation for 50 minutes at 24° C. On the other hand, similar solutions containing 2% of skimmed milk powder, soluble egg albumen or whey powder required 4 hours at 24° C. for the same degree of inactivation.

Cottonseed oil had no protective effect upon penicillin. Incubation of 1 cc. of a suspension of 100,000 units of penicillin calcium in cottonseed oil with 25 cc. of

* Hydrochloric acid (36%) . . . 16.3 cc.
Sodium chloride . . . . . 9.0 gm.
Disodium phosphate . . . . 2.0 gm.
Pepsin . . . . . 5.0 gm.
Lactic acid . . . . . 1 gm.
Amino-acetic acid . . . . . 1 gm.
Distilled water, q.s. . . . . 1000 cc.

artificial gastric juice resulted in complete inactivation of the penicillin within 30 minutes. The use of a soft elastic gelatin capsule to enclose the penicillin-oil suspension offered no protection to the drug under similar conditions.

IN VIVO STUDIES. Mongrel dogs, weighing 8 to 10 kg., were used in this investigation. Their diet consisted of single daily feedings of chopped horse meat, Gaines dog meal and milk. Water was available at all times. During an experiment the

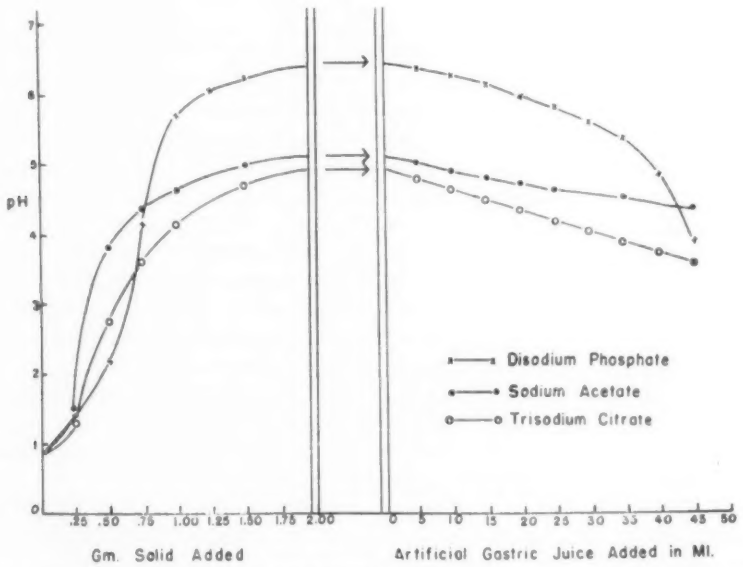
pH
Disodium Phosphate
Sodium Acetate
Trisodium Citrate
.25 .50 .75 1.00 1.25 1.50 1.75 2.00
0 5 10 15 20 25 30 35 40 45 50
Gm. Solid Added
Artificial Gastric Juice Added in Ml.

FIG. 1.—pH of artificial gastric juice following addition of various soluble alkaline salts.

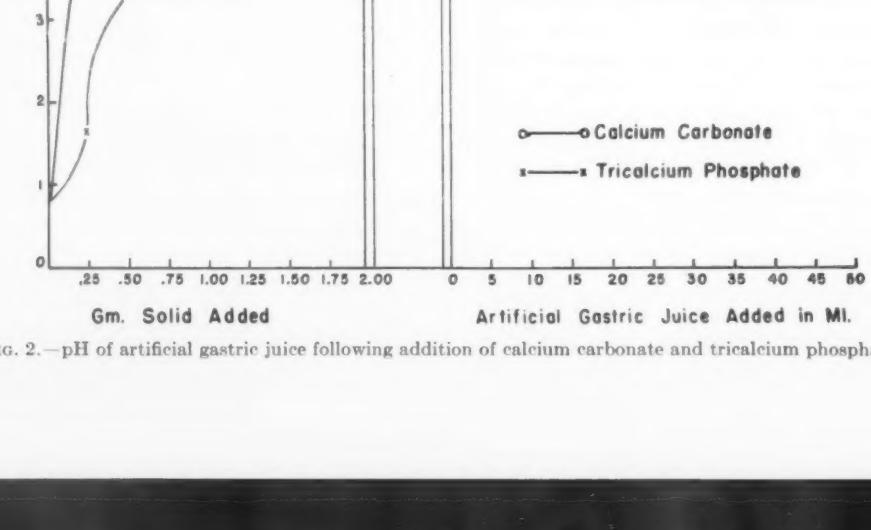
pH
Calcium Carbonate
Tricalcium Phosphate
.25 .50 .75 1.00 1.25 1.50 1.75 2.00
0 5 10 15 20 25 30 35 40 45 50
Gm. Solid Added
Artificial Gastric Juice Added in Ml.

FIG. 2.—pH of artificial gastric juice following addition of calcium carbonate and tricalcium phosphate.

dogs were kept in large metabolism cages in an air-conditioned room. The animals were fasted for 12 hours before the experiment. Prior to penicillin administration and again at the 4th hour, each animal was given 200 cc. of water by stomach tube to insure adequate urine flow.

Unless otherwise stated, penicillin calcium was administered orally in the form of compressed tablets with the protective substances as a component part. The tablets were given in single doses of 100,000 units or by repeated administration comprising an initial dose of 30,000 units and 10,000 units hourly thereafter until a total of 100,000 units was given. Penicillin calcium in oil suspension was administered orally in the form of soft elastic gelatin capsules in single doses of 100,000 units.

Serum and urine samples, taken at hourly intervals during the experiment, were assayed by a modification of the cup-plate method.[3] The minimum concentration of penicillin in the serum measured quantitatively by this test was 0.2 unit per cc.

Buffers and Antacids. In preliminary experiments each of 3 groups of dogs was pre-conditioned by the oral administration of soluble buffering salts dissolved in water in the following amounts, respectively: disodium phosphate, 6 gm.; sodium acetate, 6.5 gm.; trisodium citrate, 5 gm. Thirty minutes later 100,000 units of penicillin calcium in water was given with the following amounts of buffers, respectively: disodium phosphate, 5 gm.; sodium acetate, 3.5 gm.; trisodium citrate, 5 gm. One group of control animals without pre-conditioning received 10 gm. of lactose and 100,000 units of penicillin calcium dissolved in water. Another group received an aqueous solution of 100,000 units of penicillin calcium alone. In a second series of experiments, pre-conditioning of the animals was omitted. The penicillin and soluble buffering salts or antacids were administered in the same tablet according to the single and repeated dosage schedules outlined above. Control animals received penicillin calcium alone.

The data presented in Tables 1 and 2 indicate that oral pre-conditioning of dogs with soluble alkaline buffering salts offered no advantage over the simultaneous administration of penicillin and the buffering substance. On the other hand, the data in Table 3 indicate that repeated oral administration of soluble buffering salts or antacids in combination with penicillin resulted in high levels of penicillin in the sera of dogs. These levels were maintained as long as the administration of penicillin was continued. A mild diarrhea was observed after repeated administration of disodium phosphate, thereby detracting from the usefulness of this buffer for oral penicillin.

Early appearance of penicillin in the urine followed the rapid disappearance of the drug from the blood stream. Urinary levels ranging from 10 to 50 units per cc. were detected within 30 to 60 minutes after the administration of penicillin in combination with soluble buffers or antacids. The quantity of penicillin excreted in the urine of the dogs after repeated oral administration with such agents was 4 to 6 times that excreted after similar administration of penicillin in water.

Protein Substances. Repeated administration of soluble alkaline buffering substances and certain antacids presents the possibility of producing gastric irritation and alkalosis. In order to overcome this potential objection to the use of alkaline substances and antacids, protein materials were explored as protective agents for orally administered penicillin. Although the *in vitro* tests indicated that skimmed milk powder, soluble egg albumen and whey powder all protected penicillin against acid destruction, the urinary excretion data following the single administration of 100,000 units of penicillin with the same amounts of these 3 protein materials showed that the skimmed milk powder was much more efficient in the animal tests than soluble egg albumen or whey powder. A comparison of the data

TABLE 1.—SERUM AND URINARY CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION WITH SOLUBLE ALKALINE BUFFERING SALTS

Single Dose = 100,000 Units (With Pre-treatment)

Protective substance	Hourly serum levels (units per cc.) 1	2	3	4	5	6	7	% urinary excretion 24 hours
Disodium phosphate	0.2	0.3	0.4	0.3	<0.2	<0.2	..	26.0
Sodium acetate	0.2	0.3	0.4	<0.2	<0.2	<0.2	..	30.4
Trisodium citrate	0.3	0.2	<0.2	<0.2	<0.2	<0.2	..	23.0
Water (control)	1.6	0.7	0	0	0	0	..	4.1

TABLE 2.—SERUM AND URINARY CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION WITH SOLUBLE ALKALINE BUFFERING SALTS AND ANTACIDS

Single Dose = 100,000 Units (Without Pre-treatment)

Protective substance	Hourly serum levels (units per cc.) 1	2	3	4	5	6	7	% urinary excretion 24 hours
Sodium acetate	0.2	<0.2	<0.2	0	0	0	0	18.6
Trisodium citrate	1.6	0.8	0.3	<0.2	<0.2	<0.2	0	22.8
Calcium carbonate	1.4	1.0	0.4	0.3	<0.2	<0.2	<0.2	22.4
Tricalcium phosphate	>2.0	0.7	<0.2	<0.2	<0.2	<0.2	<0.2	26.6
Water (control)	1.6	0.7	0	0	0	0	..	4.1

TABLE 3.—SERUM AND URINARY CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION WITH SOLUBLE ALKALINE BUFFERING SALTS AND ANTACIDS

Repeated Dosage = 30,000 Units Initially and Then 10,000 Units Hourly; 100,000 Units Total

Protective substance	Hourly serum levels (units per cc.) 1	2	3	4	5	6	7	% urinary excretion 24 hours
Sodium acetate	1.2	0.4	0.4	0.7	1.0	0.4	0.8	31.1
Trisodium citrate	1.2	1.4	0.3	0.4	0.8	0.7	1.0	27.7
Calcium carbonate	0.4	0.5	0.8	0.8	1.2	0.8	0.3	46.1
Tricalcium phosphate	0.8	0.6	0.6	1.0	0.4	0.8	0.5	32.7
Water (control)	0.2	0.6	0.5	<0.2	0.3	<0.2	..	7.3

TABLE 4.—SERUM AND URINARY CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION WITH PROTEINS AND PROTEIN-CONTAINING MIXTURES

Single Dose = 100,000 Units

Protective substance	Hourly serum levels (units per cc.) 1	2	3	4	5	6	7	% urinary excretion 24 hours
Skimmed milk powder	1.2	0.8	0.2	0.2	<0.2	<0.2	<0.2	24.0
Egg albumen powder	1.4	0.4	0.3	0.2	0	0	0	15.6
Whey powder	1.5	0.5	<0.2	0	0	0	0	13.1
Water (control)	1.6	0.7	0	0	0	0	..	4.1

TABLE 5.—SERUM AND URINARY CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION WITH PROTEINS AND PROTEIN-CONTAINING MIXTURES

Repeated Dosage = 30,000 Units Initially and Then 10,000 Units Hourly; 100,000 Units Total

Protective substance	Hourly serum levels (units per cc.) 1	2	3	4	5	6	7	% urinary excretion 24 hours
Skimmed milk powder	>2.0	0.4	0.4	0.4	0.3	0.2	0.2	20.1
Egg albumen powder	0.0	0.4	0.3	0.3	0.4	0.3	0.2	6.3
Whey powder	1.0	0.6	0.7	0.6	0.6	0.5	0.3	17.1
Water (control)	0.2	0.6	0.5	<0.2	0.3	<0.2	..	7.3

Repeated Dosage = 40,000 Units Initially and Then 10,000 Units Hourly; 90,000 Units Total

Skimmed milk powder	2.0	1.4	0.8	0.5	0.7	0.5	..	32.0

TABLE 6.—SERUM AND URINARY CONCENTRATIONS OF PENICILLIN FOLLOWING ORAL ADMINISTRATION OF OIL SUSPENSIONS IN SOFT ELASTIC GELATIN CAPSULES

Single Dose = 100,000 Units

Protective substance	Hourly serum levels (units per cc.) 1	2	3	4	5	6	% urinary excretion 24 hours
Cottonseed oil	0.9	0.5	<0.2	<0.2	<0.2	0	5.0
Linseed-peanut oil	0.5	0.3	<0.2	<0.2	<0.2	0	9.0
Water (control)	1.6	0.7	0	0	0	0	4.1

presented in Tables 2 and 4 indicates that the administration of penicillin with skimmed milk powder in single oral doses produced concentrations of penicillin in the sera and urine of dogs equal to those produced by single doses of penicillin with the buffering substances and antacids. A comparison of the data in Tables 3 and 5 indicates that the repeated administration of penicillin with skimmed milk powder produced concentrations of penicillin in the sera and urine of dogs equal to those produced by the administration of penicillin with buffers and antacids in repeated doses.

No deleterious physiologic effects were observed in the animals receiving combinations of penicillin and these proteinic substances.

OILS. Oil vehicles have been reported[4] to protect orally administered penicillin against destruction by gastric acidity. In order to evaluate this method of protection, soft gelatin capsules containing suspensions of penicillin in cottonseed oil and in a mixture of linseed oil and peanut oil were administered to dogs. A comparison of the data presented in Tables 2 and 6 indicates that the administration of penicillin with oil in single oral doses produced significantly lower concentrations of penicillin in the sera and urine of dogs than those produced by single doses of penicillin with the buffering substances and antacids. However, traces (< 0.2 unit per cc.) of penicillin were detected in the sera for as long as 6 hours.

Summary. The acid inactivation of orally administered penicillin can be reduced appreciably by the simultaneous administration of protective substances. Relatively high concentrations of penicillin can be produced and maintained in the blood and urine of dogs following the oral administration of penicillin with such protective substances as soluble alkaline buffer salts, certain antacids and certain edible proteinic materials. It appears that the use of edible proteinic materials as protective agents for penicillin may permit absorption with no danger of untoward effects.

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STREPTOMYCIN IN THE TREATMENT OF CERTAIN GRAM-NEGATIVE BACILLUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

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Gram-negative bacillus infections of the meninges are infrequent when compared with other bacterial meningitides, but they are common enough to be of importance, particularly inasmuch as they are attended by high mortality rates. This report deals with 5 cases of such infections in which recovery followed streptomycin treatment.

The causative organisms were *Pseudomonas æruginosa* and *Hemophilus influenzæ* each in 2 cases and *Bacillus proteus* in 1 case. These are all gram-negative, aërobic, non-sporulating bacilli. *Ps. æruginosa* (*Bacillus pyocyaneus*) is noted for the bluish green pigment which it produces under certain circumstances. All 3 organisms occur as normal saprophytic inhabitants of the upper respiratory or the gastro-intestinal tracts of man and animals but occasionally produce severe infectious processes. *Ps. æruginosa* and *B. proteus* are commonly encountered in infections of the genito-urinary tract while *H. influenzæ* (the influenza bacillus of Pfeiffer) is most often encountered in meningitis in small children. Each of these organisms may, however, invade any portion of the body and infections due to these bacteria have been reported in the eye,[33.42.46.74] ear,[20.50.74] nasal accessory sinuses,[20.50.74] pharynx,[42.50.74] larynx,[42] endocardium,[28.42] lungs,[42.50.74] gastro-intestinal tract,[42.50.74] genito-urinary tract,[42.50.74] peritoneum,[42.50.74] liver,[42.74.77] bones[50.62.74] and joints,[42.74] meninges,[8.42.50.74] skin[25.42.74] and wounds.[48.50]

Pseudomonas Æruginosa Meningitis. *Pathogenesis.* Meningitis due to *Ps. æruginosa* usually follows direct implantation of the organism into the meninges. Occasionally meningitis due to this organism is noted in the course of a septicemia, the portal of entry for the septicemia being the genito-urinary tract or elsewhere.[26.73] Direct implantation of *Ps. æruginosa* into the meninges usually follows trauma, as in a skull fracture or a bullet wound or occurs by direct extension from an adjacent suppurative process such as an otitis media, or, it may be introduced by a contaminated spinal anesthesia or lumbar puncture procedure.[26] At least 19 cases of *Ps. æruginosa* meningitis have been reported which were considered to be the result of a contaminated spinal anesthesia or diagnostic lumbar puncture.[10.11.26.27.43.64.73.78]

Four additional cases have been reported recently following intrathecal penicillin treatment for pneumococcal meningitis. Two of them died. The source of the *B. pyocyaneus* was traced in 2 of the cases to penicillin solutions contaminated by syringes used to withdraw the drug for injection.[34a]

Treatment and Mortality. Evans[26] and Kerman *et al.*[43] reviewed the previously reported cases of *Ps. æruginosa* meningitis and found mortality rates ranging from 52 to 85% in different groups of cases. The higher mortality rates were noted in patients with other complicating conditions or where the pyocyaneus infection involved other parts of the body. In meningitis due

to this organism, without infection elsewhere, the mortality has usually been about 50%.

Since the advent of sulfonamides and penicillin, these agents have been used singly or in combination in the treatment of 21 reported cases of *Ps. æruginosa* meningitis with 15 deaths.[2,13,15,17,43,78] Treatment early in the course of the disease resulted in recovery in 4 cases treated by Wise[78] with sulfanilamide. The results in other sulfonamide treated cases, however, have not been favorable. *In vitro* experiments have shown *Ps. æruginosa* to be one of the most resistant organisms to the action of penicillin.[1,37,39] During the treatment of 400 cases of head wounds with sulfadiazine and penicillin in an Army hospital, Botterell and Magner[13] encountered 11 cases of *Ps. æruginosa* meningitis; 9 of them died of this infection despite intensive sulfadiazine and intramuscular and intrathecal penicillin therapy. Agulnik[2] reported a fatal case in a newborn infant following circumcision. His case was treated with sulfadiazine, sulfapyridine and penicillin. Penicillin has proved of little value in treatment of *Ps. æruginosa* meningitis[2,13,17] and has been shown to be without value also in the treatment of pyocyaneus bacillus infections elsewhere in the body.[18,19,48]

The *in vitro* resistance of *Ps. æruginosa* to streptomycin has been found to be of a relatively high degree when compared with an organism like *Pasteurella tularense*. The latter is inhibited by 0.15 to 0.3 units of streptomycin per ml.[36] whereas most strains of *Ps. æruginosa* require 25 to 100 units of streptomycin per ml.[14,21,37,44,60] Streptomycin has been shown to protect mice inoculated with lethal doses of *Ps. æruginosa*.[41]

Recently Cairns *et al.*[17] reported 3 cases of pyocyaneus meningitis which were treated by sulfonamides, penicillin and intrathecal streptomycin; all 3 patients died. It is noted, however, that treatment was begun late in the course of the disease in 2 of Cairns' cases—approximately 10 days after onset, while the time of institution of treatment in the third case was not given.

Case Reports. Case 1. A 14 year old white male, a known epileptic, was admitted to the hospital after a seizure. A diagnostic lumbar puncture revealed normal spinal fluid. Twenty-four hours later the temperature rose, signs of meningitis developed and a second lumbar puncture revealed purulent fluid containing 1300 leukocytes per c.mm. of which 95% were polymorphonuclears. Treatment with intrathecal and intramuscular penicillin and oral sulfadiazine was begun. Repeated lumbar punctures revealed similar cytologic findings and *Ps. æruginosa* was cultured from each specimen of cerebrospinal fluid.

No improvement followed the use of penicillin and sulfadiazine. On the 4th day penicillin was stopped and streptomycin therapy instituted. The first dose consisted of 0.05 gm. (50,000 units) intrathecally and 1 gm. (1,000,000 units) intramuscularly; this was followed by 1 gm. intramuscularly every 6 hours for a period of 7 days. He was given 2 intrathecal injections of streptomycin, 0.05 gm. each, on the following day, and thereafter 1 daily intrathecal injection of 0.05 gm. for 7 days. Sulfadiazine therapy was stopped on the 9th day of the disease because of the development of crystalluria.

The temperature returned gradually to normal during the first 4 days following institution of streptomycin therapy and clinical improvement was rapid. Daily spinal fluid examinations showed a decrease in the total number of leukocytes with an increase in the percentage of lymphocytes. Cultures of the spinal fluid revealed *Ps. æruginosa* on the 2nd and 4th days of therapy but later ones were all negative. A total of 28 gm. of streptomycin was given intramuscularly and 0.45 gm. intrathecally. Except for some fever there were no untoward reactions to the streptomycin. The patient was discharged from the hospital 20 days after onset of his disease, apparently cured and with no evidence of residual neurologic damage. The relevant findings in this case are shown in Figure 1.

Case 2. The more important findings in this case are shown in Figure 2. On the 11th day of this infant's life an ulcerated area on a congenital cervical meningocele was débrided. One day later signs of men-

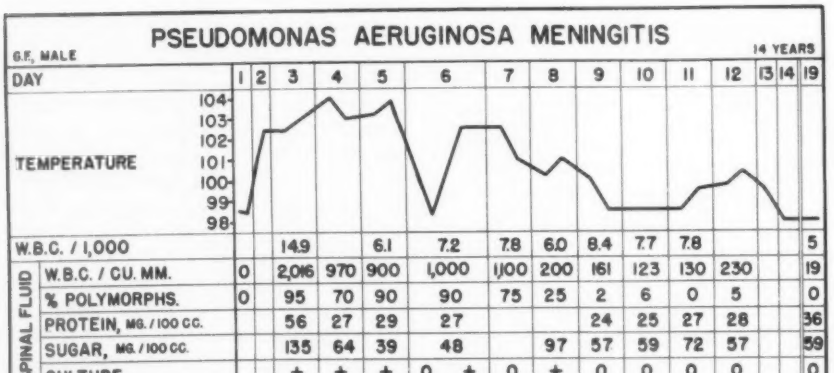

FIG. 1.—Relevant findings in Case 1. Penicillin (not shown in the figure) was given by intermittent intramuscular injections following the first rise in temperature and intraspinally after each of the lumbar punctures done on the 3rd and 4th days but was discontinued when streptomycin was begun.

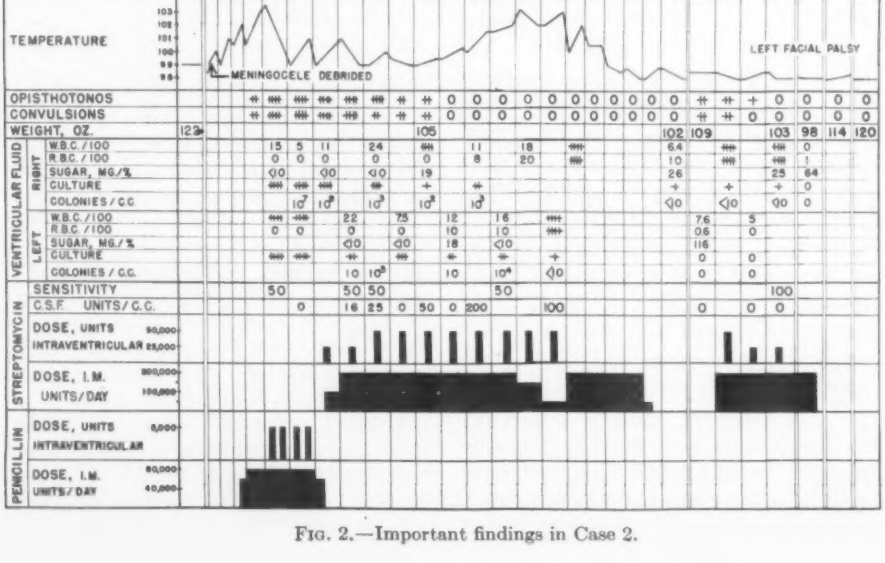

FIG. 2.—Important findings in Case 2.

ingitis developed and *Ps. æruginosa* was cultured from cerebrospinal fluid obtained by puncture of the ventricle through the fontanelle. After 4 days of ineffective treatment with intramuscular and intraventricular penicillin, that drug was stopped and streptomycin treatment started. The dose of streptomycin was 0.05 gm. every 6 hours intramuscularly and 1 intraventricular dose of 0.025 to 0.05 gm. daily in alternate lateral ventricles. Convulsions and opisthotonos, which were present before this therapy, gradually subsided and the temperature returned to normal within 5 days after institution of streptomycin therapy. From the 6th to the 10th day of streptomycin treatment the temperature showed a gradual rise and at this time intraventricular streptomycin was stopped. During this period the ventricular fluid had become somewhat bloody in appearance. Four days after cessation of the local streptomycin injections the temperature returned to normal and the cerebrospinal fluid had cleared markedly. Cultures of the fluid from the right lateral ventricle were positive for *Ps. æruginosa* through the 10th day of therapy and from the left lateral ventricle through the 27th day of treatment. A right facial paralysis appeared on that day and persisted. The infant was discharged from the hospital 8 weeks after cessation of therapy, apparently cured of the meningitis but with evidence of residual central nervous system damage. A total of 6.5 gm. streptomycin was given by the intramuscular route and 0.55 gm. by the intraventricular route.

Bacillus Proteus Meningitis. *Pathogenesis.* Meningitis due to *B. proteus* usually occurs by direct extension from an adjacent otitis media or mastoiditis.[50] It has occasionally been reported in the course of septicemia associated with infections in other parts of the body.[50] No instance of this condition occurring as a result of a spinal anesthesia or lumbar puncture procedure was found in the literature.

Treatment and Mortality. Prior to the advent of sulfonamides the mortality rate of meningitis due to *B. proteus* was over 90%. The use of sulfonamides has apparently been associated with a reduction in the mortality rate in the few cases that have been reported.[9,15,22,32,35,51,53,55,70]

B. proteus has been considered resistant to penicillin, 5 to 10 units per ml. being necessary to inhibit this organism.[1,29,37,40] Stewart,[67] however, postulated that despite the relatively high degree of resistance to penicillin, this drug may be useful in treating certain *B. proteus* infections where the penicillin can be applied topically in high concentrations. He reported the elimination of *B. proteus* from the ears of certain patients with chronic suppurative otitis media by the use of high local concentrations of penicillin in the form of a gel. On the basis of the relatively high degree of resistance of *B. proteus* to penicillin, however, the use of this drug in the treatment of meningitis due to *B. proteus* would not seem indicated.

B. proteus is moderately sensitive *in vitro* to streptomycin, being inhibited by 2 to 8 units per ml.[14,21,44,60] Streptomycin also protects chick embryos against experimental infections with *B. proteus*.[57] Keefer's[21] report on the clinical use of streptomycin includes 3 cases of *B. proteus* meningitis, 1 due to *B. proteus morgani* and the others due to *B. proteus vulgaris*. One of the patients with *B. proteus vulgaris* died; the other 2 patients survived. The time of institution of treatment in relation to the course of the disease was not stated.

Case 3. A 4 month old infant developed signs of meningitis following an operation on a congenital meningocele. Culture of the meningocele fluid revealed *B. proteus morgani*. After 6 days of treatment with penicillin given intramuscularly and into the meningocele sac and 2 days of oral sulfadiazine, there was clinical improvement and the fever was decreasing but *B. proteus morgani* was still present in cultures of the meningocele fluid.

At this point other medications were stopped and streptomycin therapy begun. Streptomycin, 0.05 gm., was injected directly into the meningocele sac once daily for 7 days and 0.1 gm. was given intramuscularly every 6 hours for 10 days. Cultures of the meningocele fluid were negative after

streptomycin treatment was begun but the temperature showed a gradual upward swing and the infant seemed worse clinically. Sulfadiazine treatment was reinstituted and the local injections of streptomycin were discontinued on the 7th day of this therapy. Four days later the temperature returned to normal and the infant seemed better clinically. Although clinical improvement coincided with the reinstitution of sulfadiazine it seems that streptomycin was chiefly responsible for the favorable result in view of the fact that cultures of the meningocele fluid became negative only after institution of streptomycin treatment. Subsidence of fever and clinical improvement also coincided with cessation of injections of streptomycin into the meningocele sac. The infant had an uneventful convalescence. A total of 4.8 gm. of streptomycin was given by the intramuscular route and 0.35 gm. by the intrameningocele route. The more important findings in this case are summarized in Figure 3.

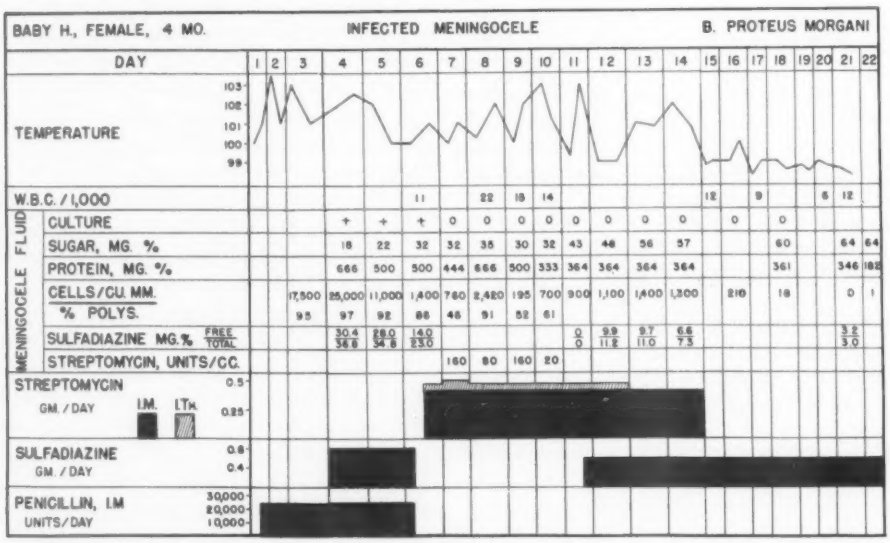

FIG. 3.—Significant data in Case 3. In addition to the therapy shown in this figure penicillin was injected into the meningocele sac daily until streptomycin treatment was started.

HEMOPHILUS INFLUENZÆ MENINGITIS. *Pathogenesis. Hemophilus influenzæ* meningitis is chiefly a disease of early childhood, 60 to 80% of the cases occurring during the first 2 years of life.[71] This age incidence may be explained, in part, by the low bactericidal properties of the blood against *H. influenzæ*, Type b, in infancy, as shown by Fothergill and Wright.[31] Pittman[58] classified some of the smooth, encapsulated strains of *H. influenzæ* into Types a and b, and later described 4 additional types on the basis of a precipitin test.[58a] Type b is the causative agent in about 95% of the cases of meningitis due to *H. influenzæ*.[4,59,65] Dochez *et al.*[23] noted that during experimental infections of chimpanzees with the virus of the common cold, when *H. influenzæ* is present in the nasopharynx of these animals, a transformation takes place from the serologically non-type-specific rough form of *H. influenzæ* to the serologically specific smooth form; during healthy periods there is a reversion to the rough form. Smooth pathogenic strains of *H. influenzæ* are encountered most often in the human nasopharynx during acute inflammatory processes while rough and presumably nonpathogenic strains are most often found in the normal nasopharynx.[34,68] Meningitis is probably acquired by invasion of the smooth pathogenic strain through the

respiratory tract by way of the blood stream.

Treatment and Mortality. The mortality of *H. influenzæ* meningitis has been reduced from 80 to 98% to 15 to 50%[3,5,20,61,66,72] since the introduction of combined sulfonamide and type-specific rabbit antiserum therapy. *H. influenzæ* has been considered relatively resistant to penicillin.[1,29,39] Straker[69] and Forgacs,[30] however, reported the isolation of smooth strains, Type b, from human meningeal infections that were sensitive to penicillin to about the same degree as the standard Oxford strain of *Staphylococcus aureus.* Hewitt and Pittman[38] tested the sensitivity of 38 strains of *H. influenzæ* isolated from cases of human infection and found them to be inhibited by 0.18 to 1.5 units per ml. of commercial penicillin (predominantly penicillin G) and by 0.05 to 0.75 unit per ml. of penicillin X. Forgacs *et al.*[30] describe improvement in a patient with *H. influenzæ* Type B meningitis on penicillin therapy alone. McIntosh and Drysdale[49] treated a case of *H. influenzæ* Type b meningitis successfully with penicillin and sulfamezathine. The organism isolated from this patient was somewhat less sensitive to penicillin than the standard staphylococcus. Landau[45] described an infant with an empyema due to *H. influenzæ* Type b which persisted despite sulfadiazine, type-specific antiserum and a thoracotomy, and apparently cleared up only after institution of penicillin irrigations of the empyema cavity. The total number of reported cases of *H. influenzæ* meningitis that were treated with penicillin are too few to warrant any conclusions concerning its efficacy in this disease.

H. influenzæ has been found *in vitro* to be sensitive to streptomycin, being inhibited by 1.56 to 15 units per ml.[6,21,38,52] Alexander and Leidy[6] found that streptomycin protects mice against experimental *H. influenzæ* infections and that this protection is not enhanced by the concurrent use of sulfadiazine. They also found that the streptomycin alone is more effective against *H. influenzæ* infections in mice than Type b specific antiserum used in conjunction with sulfadiazine. Hewitt and Pittman[38] found that streptomycin is the most effective agent in protecting mice against experimental infections with *H. influenzæ.*

Clinical in Contrast to Experimental Reports. Logan and Herrell[47] reported the use of streptomycin along with sulfonamides and specific antiserum in the treatment of 4 patients with *H. influenzæ* meningitis. The infection was apparently controlled in each of these 4 patients but 1 of them later died of hydrocephalus. Butler *et al.*[16] reported recovery of a patient with *H. influenzæ* meningitis treated with streptomycin, sulfadiazine and Type b rabbit antiserum.

Birmingham *et al.*[12] reported on the treatment of 8 cases of *H. influenzæ* meningitis—4 patients recovered, 3 died and in 1 streptomycin therapy was given up in favor of sulfadiazine and antiserum. In 1 of the patients who died, the organism became resistant to streptomycin during treatment. Nussbaum *et al.*[56] used streptomycin and sulfadiazine in the successful treatment of 3 infants below the age of 2 years. Weinstein[75] used streptomycin in 9 cases of *H. influenzæ* meningitis with 2 deaths, 1 of them due to a complicating staphylococcal infection. The prompt clinical and bacteriologic response to streptomycin therapy in the survivors was dramatic. In the 100 cases of *H. influenzæ* meningitis treated with streptomycin under the auspices of the National Research Council,[21] the mortality was 17%. In many of these patients, streptomycin treatment was begun only after other forms of treatment had failed. Alexander *et al.*[7] more recently reported 25 cases treated with streptomycin alone or combined with other agents, with 3 deaths. In 2 of her cases resistant strains of *H. influenzæ* developed during streptomycin therapy.

CASE 4. An 8 month old infant was admitted to the hospital after 1 day of fever, hyperirritability and emesis and was found

to have *H. influenzæ* Type b meningitis. Without other previous or concomitant therapy, streptomycin treatment was instituted 36 hours after the onset of symptoms. The streptomycin was given intrathecally, 0.05 gm. as an initial dose and then 0.025 gm. was given once a day for 8 days. In addition, 0.2 gm. was given intramuscularly every 6 hours for 16 days. Cultures of the cerebrospinal fluid promptly became negative and the cerebrospinal fluid sugar level returned to normal within 2 days after this treatment was started. The temperature showed a decided drop on the 4th day of treatment and this was associated with clinical improvement. There were irregular temperature rose abruptly and the signs of meningitis became more marked. On that day the cerebrospinal fluid showed an increase in the number of cells and gram-negative bacilli were noted in stained smears of the cerebrospinal fluid but cultures of the same fluid were negative. Other medications were then stopped and streptomycin was begun on the 12th day of the disease. The streptomycin was given intramuscularly, 0.2 gm. every 6 hours for 6 days, and intrathecally 0.025 gm. once daily on 3 consecutive days. During the period of streptomycin therapy the fever persisted. Signs of serum sickness were noted on the 14th day of the disease. The number of cells in the

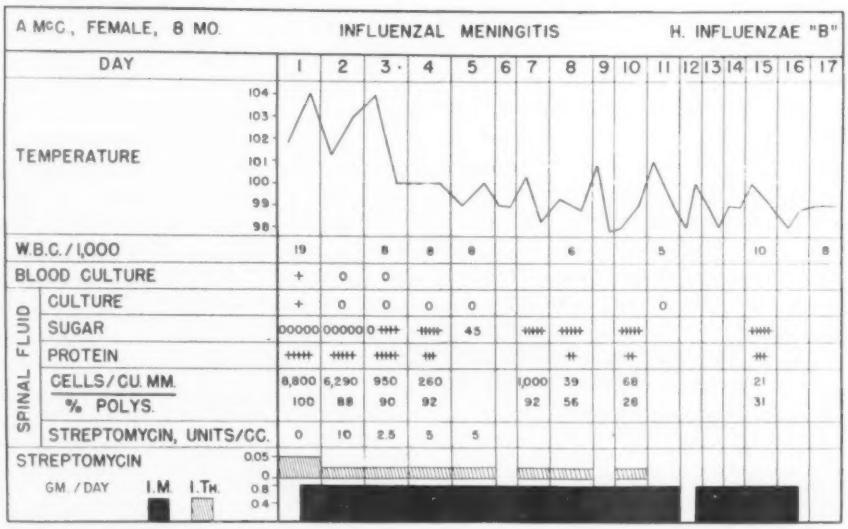

FIG. 4.—Case 4. Streptomycin was the only antibacterial agent used in this case.

elevations of temperature through the 15th day of treatment. A total of 11.6 gm. of streptomycin was given intramuscularly and 0.225 gm. intrathecally. The infant was apparently cured, without evidence of residual central nervous system damage. Figure 4 shows the relevant findings in this case.

CASE 5. An 11 month old infant was admitted to the hospital after a few hours of illness and found to have meningitis. Cultures of the blood and cerebrospinal fluid yielded *H. influenzæ*, Type b. For a period of 10 days treatment consisted of sulfadiazine, penicillin and 2 injections of specific rabbit antiserum. During this time the patient's condition seemed to improve slightly. On the 11th day, however, the spinal fluid decreased and organisms could no longer be seen in stained smears after the 1st day of streptomycin therapy. The temperature, however, did not return to normal until several days after cessation of streptomycin treatment. A total of 4.4 gm. was given by the intramuscular route and 0.75 gm. intrathecally.

SENSITIVITY OF ORGANISMS. The streptomycin sensitivity of the organisms obtained from the present cases was determined by the serial dilution tube method.[27a] The *Ps. æruginosa* isolated from Case 1, before streptomycin treatment, grew in 250 units per ml. penicillin but was completely inhibited by 25 to

50 units per ml. streptomycin on different occasions. The *Ps. æruginosa* obtained from Case 2 before streptomycin therapy was completely inhibited by 50 units per ml. streptomycin. Strains of *Ps. æruginosa* cultured from Cases 1 and 2 during the streptomycin treatment were no more resistant to streptomycin than the pretreatment strains. The *B. proteus morgani* isolated from Case 3 before treatment was completely inhibited by 50 units per ml. streptomycin. The *H. influenzæ* from Case 4 was completely inhibited by 1.56 units per ml. streptomycin. Sensitivity of the *H. influenzæ* from Case 5 was not determined since the organism seen in smear before treatment could not be grown and the earlier cultures from this patient were not available.

Streptomycin Concentrations in the Cerebrospinal Fluid and Blood. Streptomycin concentrations in cerebrospinal fluid and plasma were determined by a serial dilution tube method using strain "T" of *Klebsiella pneumoniæ* as the test organism.[27a] Streptomycin, usually 0.05 gm. (50,000 units), was given topically once daily to these patients. In Case 1, a 14 year old boy, the concentration of streptomycin in the cerebrospinal fluid 24 hours after the previous intrathecal injection of 50,000 units ranged from 3 to 25 units per ml., during the period of treatment. In this same patient, cerebrospinal fluid contained 400 units per ml. streptomycin 14 hours after the local injection of the same amount. In Case 2, a 2 week old infant, streptomycin concentrations in the ventricular fluid obtained 24 hours after a previous intraventricular injection of 50,000 units ranged from 0 to 200 units per ml., over a period of several days. The meningocele fluid in Case 3, a 4 month old infant, obtained 24 hours after a previous intrameningocele injection of 50,000 units contained from 20 to 160 units per ml. In Case 4, an 8 month old infant, cerebrospinal fluid concentrations of streptomycin ranged from 2.5 to 10 units per ml., 24 hours after the previous intrathecal injections of 50,000 units of streptomycin.

Plasma streptomycin concentrations in Case 1, obtained 6 hours after intramuscular injections of 1 gm. (1,000,000 units) of streptomycin, ranged from 6 to 13 units per ml., during the treatment period.

Untoward Effects of Streptomycin. The intramuscular administration of streptomycin was attended by no harmful effects save for the development of a subcutaneous sterile abscess at the site of repeated intramuscular injections in Case 2. The total daily intramuscular dose of streptomycin ranged from 0.2 gm. for Case 2, the newborn infant, to 4 gm. for Case 1, the 14 year old boy. The individual intramuscular doses were given every 6 hours in 1 to 4 ml. of sterile normal saline.

In Cases 1, 2 and 3, there was an elevation of temperature and an increase in cells in the cerebrospinal fluid associated with the injections of topical streptomycin. Birmingham *et al.*[12] noted an aggravation of the meningeal signs and an increase in the number of cells in the cerebrospinal fluid in 2 patients receiving intraspinal streptomycin. The increases in fever, in the number of cells in the cerebrospinal fluid and in the severity of clinical signs in Cases 2 and 3 seem definitely related to the local injections of streptomycin, since they occurred during treatment and subsided promptly after the local injections were stopped. In Case 1, there was an increase in the number of cells in the cerebrospinal fluid associated with fever on the 6th day of intrathecal therapy; the fever promptly subsided after cessation of the intrathecal streptomycin, but this also coincided with the cessation of intramuscular streptomycin.

The local injections of streptomycin were usually given daily and consisted of 0.05 gm. in 1 ml. of sterile normal saline. Cerebrospinal fluid was withdrawn into the syringe containing the streptomycin solution and the dose slowly delivered. No immediate untoward effects from these injections were observed.

Comment. In evaluating the rôle of streptomycin in the cases of *Ps. æruginosa*

meningitis, in Case 1, the clinical and bacteriologic response of the patient following institution of therapy was prompt. In this patient, the infection followed lumbar puncture and streptomycin was started relatively early in the course of the disease but only after several days of apparently ineffective penicillin and sulfadiazine therapy. In Case 2, streptomycin therapy was not begun until the 7th day of the disease after 4 days of ineffective penicillin therapy; the response of the infant to streptomycin was only gradual and there was evidence of residual damage to the central nervous system after treatment. However, the fact that the infant survived at all is remarkable inasmuch a *Ps. æruginosa* is apparently a much more virulent organism for infants than for adults. In the period of 7 days before streptomycin was begun, there was more than ample time for *Ps. æruginosa* to have inflicted severe damage on the central nervous system such as has been reported by Cairns *et al.*[17] Evidently streptomycin was responsible for the survival of this infant.

In Case 3, with *B. proteus morgani* meningitis, organisms disappeared from the cerebrospinal fluid only after the institution of streptomycin therapy, though some clinical improvement had occurred during the preceding period while penicillin and sulfadiazine were being given. Here again the recovery may be ascribed to the streptomycin.

In Case 4, with *H. influenzæ* meningitis, streptomycin therapy was attended by a prompt clinical and bacteriologic response. Since no other medications were used in this patient, streptomycin would seem responsible for the cure obtained in this infant. The rôle of streptomycin in Case 5, however, is more difficult to evaluate. In view of the failure of the patient to show an adequate response to the previous treatment, this infant, in addition to having serum sickness, probably was undergoing an exacerbation of her disease. Such a course is not uncommon in *H. influenzæ* meningitis.[72] The severity of the meningeal and cerebral symptoms, the increase in the number of cells and the occurrence of organisms in the cerebrospinal fluid all suggest that the institution of streptomycin at this point may have affected a resolution of the disease process.

The transient untoward effects of local use of streptomycin in Cases 2 and 3 and probably in Case 1, would indicate that the intraventricular and intrathecal routes, for the administration of streptomycin should be used with caution.

Conclusion. Two cases of *Ps. æruginosa* meningitis, 1 case of *B. proteus morgani* meningitis and 2 cases of *H. influenzæ* Type b meningitis were successfully treated with streptomycin. Streptomycin, administered by the intrathecal and intramuscular routes would seem to be the drug of choice in the treatment of meningitis due to these gram-negative bacilli.

Case 1 was from the Fifth Medical Service (Boston University), Cases 2 and 3 were from the Neurosurgical Service and Cases 4 and 5 were from the Pediatrics Service. We are indebted to the physicians and surgeons of these services for the privilege of studying these cases.

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THE SIGNIFICANCE OF THE MYELOID MATURATION CURVE IN MATERIAL ASPIRATED FROM THE STERNAL BONE MARROW

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The differential count of material aspirated from the sternal marrow cavity offers a method for the study and evaluation of cytologic changes which occur in diseases involving the hematopoietic system. The data upon which this study was based consist of a series of differential cell counts of the bone marrow from 165 individuals, 159 of whom were afflicted, either primarily or secondarily, with a variety of diseases affecting the hemo-lympho-poietic system. The bone marrow aspiration technique justifies itself by making available data upon which a more specific diagnosis may be made and by providing a method for further inquiry into the causes and nature of disorders affecting the formation of blood cells.

Methods. I. The Technique of Sternal Puncture and the Preparation of Bone Marrow for Examination. All samples of bone marrow were obtained by puncture of the sternal marrow cavity in the midline at the level of the third rib. Less than 0.5 cc. of marrow pulp was forcibly aspirated. Coverslip preparations were made, stained with Wright's stain, and differential counts of 300 to 500 marrow cells were made from many suitable fields.

II. Criteria of Cell Types. (*a*) *The Myeloid Series.* The classification by which the cells of this series were identified is that described by Naegeli,[9] and further defined by Nordenson,[10] and Wintrobe.[22]

(*b*) *The Erythroid Series.* The descriptions of the cells of this series also follow those given by Naegeli[9] and the differential criteria of classification are consistent with those described by Segerdahl,[19] Nordenson,[10] Schulten,[17] and Wintrobe,[22] with the exception that Schulten refers to the megaloblast of Naegeli as a pro-erythroblast. In this paper, the erythropoietic cells were differentiated into the *megaloblast*, the earliest definable form of the erythroid series, the *erythroblast*, the intermediate form containing a "spoke wheel" nucleus, and the *normoblast*, the smallest form containing a dense pyknotic nucleus and hemoglobinized cytoplasm.

(*c*) *The Lymphocytic, Monocytic and Plasma Cell* series were classified according to the criteria of Naegeli.

III. Analysis of Data. All numerical data were analyzed by the use of standard statistical methods.[2]

The normal series of bone marrow differential counts consists of 1 analysis from each of 6 normal individuals. The *means* and *standard deviations* for each cell type are shown in Table 1. Those figures which are significant in terms of their *standard errors* are *italicized* and are used as a basis for judging the unusual occurrence of any given type of cell found in the bone marrow in conditions of disease. Variations of more than 3 *sampling errors* from the *mean* of the normal group are considered to have probable diagnostic significance, and variations of 2 to 3 sampling errors are considered to have only possible diagnostic significance.

A selection of authors whose techniques agree with and whose data resemble those presented here is given in Table 2. For purposes of comparison the differential counts of the myeloid series were expanded to a base of 100.

The clinical series of bone marrow differential counts were tabulated on 159 patients suffering from a variety of diseases which for reasons of simplification were classified into 24 categories. The calculated *mean* for each cell type for each disease is listed in Table 3.

Those values which are significant in terms of their *standard errors* and which deviate more than 3 *sampling errors* from the *mean* of the normal group are *italicized* and those which deviate between 2 and 3 *sampling errors* are indicated with **bold** type. The *italics* in parenthesis indicates significant deviations from the normal but the *standard errors* of these values are so great, possibly due to the smallness of the sample under consideration, that they are of less significant value for comparative purposes. The values which are in ***bold italics*** are of diagnostic significance because of their magnitude and because of the scarcity of their occurrence in the normal bone marrow, the unusual exception being the value of 15.9 for unclassified cells (primitive histiocytes) which occurs in monocytic leukemia.

BRIEF REVIEW OF THE LITERATURE. According to the literature, the differential count of the bone marrow has been regarded as of definite value in:

1. *The Differential Diagnosis of the Anemias.* Reich[15] described several cases in which he was able to differentiate the variety of anemia by means of the differential count of the bone marrow. Isaacs[3]

TABLE 1.—MEAN VALUES OF THE DIFFERENTIAL CELL COUNTS OF THE BONE MARROW FROM 6 NORMAL INDIVIDUALS

		Mean	±	Standard error of the mean		Standard deviation (σ)	±	Standard error of the σ
Myeloblasts		1.0	±	0.4		0.9	±	0.3
Promyelocytes		*4.0*	±	*0.6*		*1.4*	±	*0.4*
Myelocytes	N	*10.2*	±	*1.3*		*3.1*	±	*0.9*
	E	*0.8*	±	*0.2*		*0.5*	±	*0.1*
	B							
Metamyelocytes	N	*13.2*	±	*1.1*		*2.7*	±	*0.8*
	E	*1.3*	±	*0.2*		*0.4*	±	*0.1*
	B							
Polymorphonuclear leukocytes	N NF	*24.6*	±	*3.9*		*9.7*	±	*2.8*
	N F	*10.0*	±	*1.5*		*6.2*	±	*1.8*
	E	0.3	±	0.1		0.3	±	0.1
	B							
Total granulocytic series					**65.4**			
Lymphoblast								
Early lymphocyte								
Lymphocyte		*10.3*	±	*1.7*		*4.4*	±	*1.2*
Plasma cell		*1.5*	±	*0.5*		*1.3*	±	*0.4*
Total lymphocytic series					**11.8**			
Monoblast		0.2	±					
Promonocyte		0.2	±					
Monocyte		0.5	±					
Total monocytic series					**0.9**			
Megaloblast		0.6	±	0.8		1.4	±	0.6
Erythroblast		*8.0*	±	*2.5*		*6.2*	±	*1.8*
Normoblast		*12.0*	±	*2.6*		*6.5*	±	*1.9*
Total erythrocytic series					**20.6**			

Values which are significant in terms of their standard errors are italicized.

TABLE 2.—A COMPARISON OF DATA FROM SELECTED AUTHORS SHOWING THE MATURATION CURVE OF THE MYELOID SERIES OF NORMAL BONE MARROW OBTAINED BY ASPIRATION (VALUES EXPANDED TO A BASE OF 100)

	No. cases	Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	P M N NF	P M N F
Segerdahl[19]	52	1.9	2.1	24.2	23.2	15.5	33.1
Isaacs[3]	11	3.4	..	5.4	40.7	25.3	25.3
Scott[18]	6	2.6	6.5	22.0	22.5	23.0	23.5
Plum[12]	40	2.5	4.2	13.8	13.9	50.3	15.3
Wintrobe[22]	..	3.1	7.8	21.2	33.8	34.2	
Osgood[13]	..	1.9	2.2	22.2	23.0	13.3	37.4
Lucia and Hunt	6	2.0	6.1	16.7	22.1	37.4	15.7

TABLE 3.—THE CALCULATED MEAN VALUES OF THE CELL TYPES IN BONE MARROW OBTAINED BY ASPIRATION FROM NORMAL AND DISEASED INDIVIDUALS

	No. cases	Myelo-blasts	Promyelo-cytes	Myelocytes			Metamyelocytes			Polymorphonuclear cells			Lymphocytic series										
				N	E	B	N	E	B	NF	F	E	B	Lymphoblasts	Prolymphocytes	Lymphocyte	Plasma cells	Monoblast	Promonocyte	Monocyte	Megaloblast	Erythroblast	Normoblast	Primitive cells	Unclassified cells	Giant cells
1. Normal	6	1.0	4.0	10.2	0.8	..	13.2	1.3	..	24.6	10.0	0.3	..	..	..	10.3	1.5	0.2	0.2	0.5	0.8	8.0	12.0	0.9	..	0.3
2. Leukemia: Lymphatic	8	0.8	3.9	7.9	1.2	..	9.3	0.8	0.1	7.5	2.8	0.4	0.1	0.8	*12.8*	*34.8*	1.3	..	..	0.3	0.3	3.4	9.8	1.4	..	
3. " Myeloid	7	*(4.0)*	*(14.6)*	**17.7**	1.2	..	12.8	1.5	..	21.7	6.6	0.8	1.2	..	..	2.1	0.5	0.6	..	0.3	0.6	3.3	6.5	3.2	0.8	0.2
4. " Monocytic	3	..	3.9	3.4	4.3	0.3	2.0	1.2	..	4.7	1.5	0.7	..	..	0.3	6.5	*23.4*	0.1	2.3	4.1	2.1	***15.9***				
5. Lymphosarcoma	2	0.5	1.1	5.3	0.2	..	7.3	0.4	..	6.3	1.2	0.3	..	0.5	3.2	*55.7*	..	..	*5.8*	*14.1*	0.2	1.9	13.0	0.7		
6. Plasma cell myeloma	7	1.4	2.0	11.8	0.9	..	7.3	1.3	..	9.5	4.0	1.3	..	..	4.4	16.9	*13.6*	..	..	2.4	0.7	0.8	6.3	13.3	1.8	3.1	
7. Leukemoid reaction	4	1.1	4.0*	10.6	1.2	..	11.4	2.1	..	18.0	11.0	8.9	..	..	0.5	14.6	1.3	..	..	0.3	0.9	2.5	10.1	0.6	1.3	
8. Anemia: Pernicious	7	1.6	4.8	14.7	1.7	..	13.5	0.6	..	14.4	7.2	0.6	0.2	..	..	7.4	0.7	..	..	0.2	5.7	9.9	14.2	1.8	0.7	
9. " Secondary	15	1.2	3.3	11.2	1.8	..	12.7	0.6	..	14.8	3.9	0.5	..	..	0.2	7.4	2.3	0.1	..	0.3	0.8	7.4	29.4	1.3	0.2	0.1
10. " Hemorrhagic	5	1.9	5.5	10.8	1.8	..	17.3	1.3	..	14.9	9.0	0.3	0.4	..	0.1	8.5	0.7	..	..	0.6	1.7	9.4	13.5	1.5	0.3	0.4
11. " Hemolytic	3	0.9	4.4	7.1	1.0	..	11.8	0.8	..	13.1	4.1	..	..	..	..	9.6	2.0	..	..	0.1	1.7	16.6	24.5	2.1		
12. " Myelosclerotic	4	1.0	4.0	10.4	2.0	0.1	13.2	2.4	0.7	25.6	8.1	0.5	0.3	..	0.3	6.6	0.9	..	1.2	1.1	1.1	5.0	12.3	3.1		
13. " Cause unknown	15	1.3	4.0	11.8	1.7	0.1	11.9	1.1	..	16.1	4.9	0.6	0.2	..	0.1	9.5	1.2	..	..	0.2	2.4	10.4	20.2	1.5	0.2	0.2
14. " Miscellaneous	8	1.4	3.8	12.1	0.8	..	14.2	1.0	..	17.3	7.0	0.5	..	..	..	11.8	1.0	..	0.2	0.4	0.7	5.9	19.8	0.8	0.8	
15. Hodgkin's disease	12	1.5	6.0	12.3	1.9	..	17.0	1.2	..	21.9	6.4	0.8	0.1	0.1	..	8.7	1.6	0.2	..	0.2	0.3	5.2	12.8	1.4	0.1	
16. Polycythemia	3	1.4	2.6	8.4	1.1	..	11.2	1.2	..	18.3	18.1	0.2	0.5	..	..	7.0	1.0	..	..	1.7	0.2	7.4	17.9	1.9	..	0.1
17. Thrombopenic purpura	5	1.9	5.0	10.8	0.8	..	12.7	0.5	..	16.7	9.0	0.4	0.2	..	..	13.1	0.8	..	..	0.7	0.2	8.2	17.4	1.4		
18. Cong. hemolytic icterus	4	1.3	3.4	8.2	0.9	0.1	9.1	1.4	..	11.4	7.6	0.1	..	..	..	6.0	0.4	..	..	0.6	1.9	16.4	30.2	0.9		
19. Splenomegaly	10	1.5	5.4	11.4	2.1	..	11.9	1.2	..	17.7	7.3	0.7	0.1	..	..	8.3	0.9	..	..	0.9	0.3	7.4	20.2	0.8	1.0	0.1
20. Banti syndrome	3	2.8	*(3.0)*	9.3	1.8	..	11.9	1.4	..	11.3	4.8	0.1	0.1	..	0.4	10.4	0.8	..	..	0.8	2.3	13.1	23.6	1.8		
21. Boeck's sarcoid	4	1.6	4.5	13.6	2.9	..	13.1	0.5	..	22.2	11.6	0.9	0.1	..	..	4.8	0.6	..	..	0.3	2.1	8.1	9.7	1.2	2.4	
22. Lupus erythematosus	2	2.3	*(19.3)*	14.1	0.5	..	**18.7**	0.3	..	19.5	7.1	..	..	1.0	8.3	2.6	..	..	..	0.3	1.5	1.6	10.4	1.6		
23. Dorso-lateral sclerosis	4	1.5	.5	8.0	**2.0**	..	12.3	1.9	0.1	26.1	11.3	0.5	0.4	..	..	7.8	0.8	..	..	0.7	0.8	5.3	**14.3**	1.5	0.5	
24. Cirrhosis of liver	3	0.8	7.1	13.6	0.3	..	18.1	0.3	..	23.2	10.0	0.9	0.7	..	..	6.5	0.9	..	..	0.7	0.5	5.6	8.9	1.6		
25. Other diseases	15	1.6	5.8	12.0	1.0	..	16.5	1.1	0.1	20.3	8.4	0.6	0.1	..	..	6.2	1.2	0.1	..	0.3	0.4	7.0	16.1	1.1	..	0.1

* Includes promyelocyte eosinophil 0.1.

observed a phenomenon in the bone marrow which he designated the stage of "block of growth." He suggested that the anemias may be classified in terms of the stage at which inhibition of the precursors of the red blood corpuscles occurs. For example, inhibition of development at the level of the primitive blast stage would be considered characteristic of aplastic anemia; inhibition of development at the megaloblast stage would be considered characteristic of pernicious anemia, many of the macrocytic anemias, and in some instances of cirrhosis of the liver; and inhibition or delay of development at the level of the normoblast stage would be observed frequently in leukemias and infections.

Our observations, on the other hand, indicate that the only outstanding diagnostic feature of the bone marrow with reference to the erythropoietic series is the presence of a predominance of megaloblasts in pernicious anemia.

2. *Establishing a Diagnosis of a Disorder of the Hematopoietic System.* The differential count of the bone marrow is believed to have diagnostic significance in subleukemic monocytic leukemia and in aplastic anemia;[23] aleukemic lymphatic leukemia;[15] aleukemic leukosis;[1] Gaucher's disease, certain carcinomas, and malaria;[21] anemias due to deficiency of liver complex, myelomatosis, and subleukemic and leukopenic leukosis;[18] in doubtful or obscure anemias;[1,4,23] and in doubtful or obscure leukemias.[1,4,15,18,21,23]

3. *Confirming a Diagnosis of a Disorder of the Hematopoietic System.* This has been demonstrated in multiple myeloma, aleukemic leukemia, and Banti's disease;[23] agranulocytosis, aplastic anemia, polycythemia, hemolytic jaundice, sprue, pernicious anemia, leukopenic infectious monocytosis, and leukemia;[21] and in kala azar and relapsing fever.[18]

Discussion. 1. *The Normal Bone Marrow Differential Count.* A comparison of the *mean* normal values of the bone marrow differential count found in the publications of a large group of authors, as well as a selected group (Table 2) chosen because of similarities in techniques, showed such disparity that it was considered inadvisable to refer for interpretation any potentially pathologic bone marrow counts to the "normal" values already published. In other words, the interpretation of a bone marrow differential count must be referred to a set of standard *mean* values determined by a given observer on a series of normal individuals. Under the best of circumstances when a small amount of marrow pulp is forcibly aspirated and then examined, the differential count will give a quantitative approximation of the qualitative cytologic variation of the marrow *in situ*. An attempt to compare, and use as a base line, the curves of myeloid maturation calculated from the data of various investigators serves no useful purpose because they are not comparable. The differences in actual numbers for the various cell types, as found in the literature, may be due to: (1) differences in nomenclature and definition of cell types; (2) the degree of dilution of the marrow pulp with blood; (3) nutritional and environmental changes to which the population under analysis is subjected; (4) statistical errors induced by small sampling; and (5) variations of chance.

II. The Bone Marrow Differential Count in Disease. (*a*) *Pernicious Anemia.* In pernicious anemia there is a marked absolute increase in megaloblasts during relapse and a rapid conversion of megaloblastic to normoblastic marrow after specific treatment.[1,3,7,10,21] The shift in the curve of maturation in pernicious anemia, from megaloblastic to normoblastic following treatment with liver substance is described by Limarzi and Levinson,[7] who noted an unusual morphologic cell change involving multipolar mitosis and the formation of multinucleated erythroblasts. According to Nordenson,[10] the disturbance in the maturation process also involves the myeloid series. The promyelocytes and myelocytes become larger than usual and their nuclei become swollen and porous (dyspoietic). The chromatin

framework is condensed and knotted instead of being evenly distributed throughout the nucleus. "The degenerative changes are almost pathognostic for pernicious anemia, though a suggestion of similar changes has been observed in lymphogranulomatosis, granulocytopenias and severe anemias in cancer." Schleicher[16] considered the morphologically altered reticulum found in pernicious anemia to be of diagnostic significance for that disease and its biologic variations. He also stated that, "Under specific therapy or spontaneous remission the diseased reticulum seems to become depleted and thus proliferation of pathologic stem cells ceases. The maturation process of the promegaloblast is apparently promoted, however, developing into a normoblast-like erythroblast rather than [a] true normoblast."

Our data on 7 cases of pernicious anemia show a significant* increase in megaloblasts with evidence of myeloid irritation and a slight shift of the myeloid curve to the left (Table 3).

(*b*) *Plasma Cell Myeloma.* Vogel *et al.*[21] noted in plasma cell myeloma a definite increase in plasma cells "ranging from a true plasma type to a more undifferentiated one" and described a case in which 20% of the bone marrow cells were of these varieties.

Our 7 cases of plasma cell myeloma showed a mean incidence of 13.6% plasma cells. This value is statistically significant. An incidence of plasma cell values beyond 6% in the bone marrow may be considered diagnostic of myelomata.

(*c*) *The Leukemias.* (1) In *myeloid leukemia* the myeloblasts are present in appreciable numbers in the acute form and myelocytes predominate as the disease progresses to the chronic form.[1,10,20] In our series the myeloid maturation curve shifts to the left in the direction of greater numbers of the earlier forms of the myeloid series (Table 3), the greatest change being found in the promyelocytes followed by the myelocytes and lastly the myeloblasts. The calculated values for myeloblasts and promyelocytes show a definite deviation from the normal but their significance is diminished because of the smallness of the sample. Similar changes in each cell type may be found in conditions other than myeloid leukemia and therefore these values are not absolutely diagnostic of leukemia but only confirm the clinical impression of leukemia.

(2) In *lymphatic leukemia,* Nordenson[10] differentiates the acute form from the chronic form by the appearance of increased numbers of lymphoblasts in the former and of lymphocytes in the latter. According to Vogel *et al.*[20] the bone marrow differential in this condition shows a definite increase in lymphocytes, the precise morphologic characteristics of which were not easily definable.

In *lymphosarcoma,* Dameshek[1] observed the presence of many lymphoblasts, while Vogel *et al.*[21] found the bone marrow differential to be normal.

Our data show a significant increase in lymphocytes in both lymphatic leukemia and lymphosarcoma. Early forms of the lymphocyte appear to be definitely increased in lymphatic leukemia, but our series of cases is too small to determine the statistical value of this increase. A large number of lymphocytes occurring in the bone marrow may be considered indicative of lymphosarcomatous metastases.

(3) In *monocytic leukemia,* Dameshek[1] reported a marked increase in both monocytes and histiocytes. Vogel *et al.*[20] reported 1 case of monocytic leukemia in which there were 45% "monocytic myeloblasts," and 22% monocytes.

Differentiated monocytes appear so infrequently in the normal bone marrow that any definite increase probably has diagnostic significance. Monocytic leukemia is characterized by a marked hyperplasia of primitive reticulum cells. In our

* Significance in this instance is based on comparing the *mean* of the megaloblasts in the disease group (pernicious anemia) with the *mean* of the megaloblasts for all groups (1 ± 0.1), since the *mean* for megaloblasts in the normal group (0.6 ± 0.8) was not a significant figure in terms of its *standard error* as shown in Table 1.

small series of 3 cases not only were the differentiated monocytes present in large numbers, but the promonocytes, monoblasts and primitive unclassified cells (histiocytes) also appeared in appreciable numbers.

(*d*) In *secondary anemias*, Nordenson[10] found in bone marrow that myeloid stimulation predominated and that it was accompanied by normoblastic erythropoiesis. Dameshek[1] reported marked erythroblastic responses in hemolytic anemia. In the anemia secondary to nutritional disturbances, Vogel *et al.*[21] observed normal leukopoietic responses associated with moderate degrees of erythropoiesis.

Our group of 15 cases of secondary anemia includes anemias due to chronic arsenic poisoning, idiopathic hypochromemia with achlorhydria, nutritional disturbances, dysthyroidism, subacute bacterial endocarditis, carcinomatous metastases and senility. In general, there is a definite increase in normoblasts in any condition where there is active regeneration of red blood corpuscles. These findings also occur in the hemolytic and hemorrhagic anemias and are especially noted in the anemia of congenital hemolytic icterus. A general reduction in the cellularity of the marrow is found in aplastic, myelosclerotic and myelophthisic anemias.

(*e*) In *polycythemia*, the reports of bone marrow counts vary from those showing a generalized proliferation of the megakaryocytes, erythroblasts and myeloblasts (Dameshek[1]), to those showing changes characteristic of an excess of maturation factor (Vogel *et al.*[21]). Our observations, as do those of Nordenson,[10] fail to reveal any characteristic findings.

(*f*) *The marrow in other diseases* not classified as disorders of the hematopoietic system. Vogel *et al.*[21] classified such diseases according to the findings in the bone marrow as (1) those producing a myelopoietic shift to the right, (2) those producing a myelopoietic shift to the left, and (3) conditions in which the myeloid maturation curve remains normal. Some diagnostic entities are found to occur in more than 1 category, indicating that the presence or absence of a shift in the myeloid maturation curve in these disorders is of little or no significance. In these conditions, the shift in maturation which occurs in the bone marrow is a characteristic of the reactivity of the patient to a given disease rather than a characteristic of the disease itself. Therefore, positive alterations in the bone marrow differential counts may be of value only in corroborating a clinical diagnosis.

The differential count of material aspirated from the sternal bone marrow in this group of diseases reveals no findings of diagnostic significance except for those conditions discussed below.

Lupus Erythematosus. Dameshek[1] described 1 case of acute lupus erythematosus in which the bone marrow was hyperplastic and there were small areas of necrosis "affecting particularly the leukopoietic elements. There was an increase in reticulum cells. . . ."

Our data on 2 cases of lupus erythematosus showed bone marrow differential counts characteristic of the "leukemoid" reaction with a definite shift to the left of the myeloid series especially affecting the promyelocytes.

Sarcoidosis. Dressler[8] was able to diagnose sarcoidosis on the basis of the cellular characteristics of material obtained by bone marrow puncture. We have been unable to confirm these findings in our study of 4 cases.

The Banti Syndrome. According to Limarzi *et al.*,[6] the earliest stage of the Banti syndrome is characterized by myeloid hyperplasia; later there is maturation arrest of the myeloid and megakaryocytic tissue, and in the final stages, identified by cirrhosis of the liver, the bone marrow shows marked immaturity in erythropoiesis as well as the above cited changes in myelopoiesis. In our study of the Banti syndrome, the bone marrow revealed no significant deviations from the normal.

III. The Significance of Eosinophils

Occurring in the Bone Marrow. Vogel *et al.*[21] reported bone marrow differential counts on 15 cases of splenomegaly to be essentially normal with an increase in eosinophilic myelocytes in a few cases. In Hodgkin's disease he found a slight myeloid shift to the left and in some cases an increase in eosinophils and reticulum cells.

Our data showed an increase of eosinophils in Hodgkin's disease, splenomegaly, dorso-lateral sclerosis and in sarcoidosis; all of them chronic diseases characterized by sclerotic or fibrotic changes in the bone-marrow or hematopoietic tissues. Eosinophils appear in relatively increased numbers in allergy, certain protozoan infestations, and in the repair phase following infections.

IV. The Significance of the Myeloid Maturation Curve. The myeloid maturation curve may be defined as the curve produced by drawing a line through the average numbers of the different cell types of the myeloid series from the myeloblast through the successive stages of maturation to the polymorphonuclear filamentous cell.* Under ordinary circumstances, the curve normally progresses by relatively regular increments to a peak at the polymorphonuclear non-filament stage and tends to drop appreciably thereafter. Thus the values obtained for the myeloid maturation curve in material obtained from the sternal bone marrow may be closely correlated with the Arneth or Schilling indices in the peripheral blood. The only condition producing a consistent shift of the myeloid maturation curve to the left in the bone marrow is myeloid leukemia. On the other hand the "leukemoid" reaction affects the entire maturation curve, and quite frequently there is a tendency to shift toward the right. In myeloid leukemia the curve is definitely skewed to the left, while in the "leukemoid" reaction the curve closely resembles the normal with the exception that the total relative number of myeloid cells is greatly increased. The "leukemoid" reaction occurs in chronic infections, such as sepsis and in some cases of tuberculosis; in lupus erythematosus, sarcoidosis and dorso-lateral sclerosis; in some cases of cirrhosis of the liver and neoplasms; and as a response to irritation of the myelopoietic tissue in the course of accelerated erythropoiesis.

It was our hope that the differential counts of material obtained by aspiration of the bone marrow would give information pathognomonic of certain disease entities involving the hematopoietic system. A careful analysis of the data presented shows that this laboratory technique adds only corroborative evidence of the presence of a given disease and only in unusual circumstances can a definite diagnosis be made based purely on the cytologic changes in the bone marrow. Therefore, observations made on the cytologic alterations of the bone marrow in any given case must be interpreted in terms of the clinical findings.

Summary. 1. Differential counts of material aspirated from the sternal bone marrow were performed on 6 normal individuals and on 148 diseased individuals whose illnesses were classified into 24 categories.

2. The *mean* normal values which were used as a base line for determining significant deviations from the normal compare favorably with data obtained from other observers whose techniques were comparable.

3. Significant deviations from the *mean* normal values were noted in myeloid, lymphatic and monocytic leukemias, lymphosarcoma, plasma cell myeloma and in conditions causing active regeneration of erythropoietic tissue such as pernicious anemia, secondary anemia and congenital hemolytic icterus.

4. Diagnostic significance may be attached to the presence of increased numbers of monocytes, megaloblasts, or plasma cells in material aspirated from the sternal

* For purposes of calculation of the myeloid maturation curve, the absolute values for the varicus cell types were based upon 100 myeloid cells.

bone marrow. These cell types occur so rarely in normal bone marrow that their presence, in all probability, indicates an important lead to the accurate diagnosis of the limited number of diseases in which they occur. A marked increase in monocytes and in primitive unclassified cells (histiocytes) occurs in monocytic leukemia; a similar increase in megaloblasts is diagnostic of pernicious anemia; appreciable increases in lymphocytes occur in lymphatic leukemia and in lymphosarcoma; and an incidence of plasma cells greater than 6% in the bone marrow may be regarded as diagnostic of plasma cell myeloma.

5. An increased number of normoblasts indicates stimulation of erythropoiesis but is not diagnostic of any specific disease condition.

6. An increase of eosinophils occurs in the bone marrow in allergy, certain protozoan infestations, in the repair phase following infections, and in diseases characterized by fibrotic alteration of the marrow.

7. In myeloid leukemia there is a definite shift of the myeloid maturation curve to the left, the promyelocytic cells showing the greatest change. These findings are confirmatory but not specifically diagnostic of myeloid leukemia.

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TREATMENT OF PERNICIOUS ANEMIA WITH SYNTHETIC L. CASEI FACTOR

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With the synthesis of the *L. casei* factor from liver in 1945,[1] this newest member of the water-soluble vitamins became available in adequate supply for clinical trial. The exact relationship of this material to the folic acid from spinach of Mitchell, Snell and Williams,[19] by which name it is popularly known, vitamin Bc,[23] vitamin M,[10,11,12] the norite eluate factor,[16,26] *S. lactis* R and *S. lactis* U factors,[31] remains to be established. Certain similarities are apparent in their biologic characteristics. Their close chemical relationship is indicated in the recent report of the chemical structures of the *L. casei* factors from liver and an *S. lactis* factor,[2] which differ from each other only in that the former contains a molecule of glutamic acid. This problem has been fully discussed elsewhere, and will not be considered further here.[4,6,22,34]

The observation that the leukopenia produced in monkeys and rats on deficient diets could be corrected by the *L. casei* factor[12,15,17] led to its clinical trial in patients with leukopenia from various causes. Most reports on the treatment of granulocytopenia are unfavorable, but Watson *et al.*[33] report good results in cases of granulocytopenia due to Roentgen therapy. Berry *et al.*[3] report leukocyte responses in 5 malnourished patients with leukopenia.

The similarity between the sprue syndrome in man and vitamin M deficiency in monkeys which had been successfully treated by Day *et al.*[12] with folic acid led Darby[7,8,9] and Spies[28,30] and their collaborators to exhibit the newly available synthetic *L. casei* factor in a number of patients with sprue with excellent results, including a favorable hematologic response.

Darby and Jones[7] treated 2 cases of sprue with macrocytic anemia with 15 mg. synthetic folic acid intramuscularly daily. Glossitis disappeared on the 4th day. Reticulocyte peaks of 15.3% and 16.1% were attained on the 9th day and 4th day respectively. Darby, Jones and Johnson[8,9] reported similar results in 3 more cases of sprue with reticulocyte peaks of 11 to 43% on the 6th to 8th days of treatment with 15 mg. folic acid intramuscularly daily. Hemoglobin, red cell and white cell count rises followed. The diarrhea and impaired absorption showed improvement. They calculated[9] that the maintenance dose of folic acid would be 0.1 to 0.2 mg. daily, while therapeutic dosage would be in the vicinity of 1 mg. per day. This deduction was based not on clinical study but on the fact that the chick requirement for the *L. casei* factor was one-fifth to one-tenth that for riboflavin. Spies *et al.*[30] treated 3 patients with sprue and macrocytic anemia with folic acid, 100 mg. twice daily by mouth, with a reticulocyte response on the 4th to 5th day, reaching a peak of 17.2 to 22.7% on the 6th to 7th day. Three other patients who did not respond significantly to 5 mg. daily, responded fairly satisfactorily to 10 mg. daily. Similar hematologic and clinical improvement was reported by Spies and his group[28] in 9 such patients, who were treated with 10 to 200 mg. folic acid daily.

* Since this paper was submitted, we have treated one patient with Addisonian pernicious anemia with 1.25 mg. folic acid daily by mouth. The initial hemoglobin was 50 per cent and the red count 2.3 million. On the eighth day of therapy, a reticulocyte peak of 5.0 per cent was attained. No second response occurred when the dosage was increased to 2.5 mg. On the twenty-fourth day, the hemoglobin was 72 per cent and the red count 3.7 million.

Crude folic acid concentrates (using the term generically, not in the strict sense of the compound of Mitchell, Snell and Williams[19]) had been tried in 1944 by Castle *et al.*[5] and by Moore and his associates[20] in the treatment of pernicious anemia without response. Sharp and his associates[25] in 1944 treated 10 patients with refractory anemia, including pernicious anemia, with vitamin Bc yeast concentrate, with increase in red cell volume; but Watson[33] had found it ineffective in "refractory anemia." In November 1945, Spies and his co-workers[27] reported the first favorable response of patients with macrocytic anemias to synthetic folic acid. They treated 5 patients with 20 to 50 mg. daily intramuscularly, while the diet was controlled to exclude meat and meat products. In 3 to 4 days definite subjective improvement was noted. The reticulocyte response began on the 3rd to 8th day and reached a peak of 6.5 to 14.5% between the 5th and 10th days. Another group of 4 patients was treated with 50 to 100 mg. daily by mouth with similar effect. Vilter, Spies and Koch[32] reported the successful treatment of 14 cases of macrocytic anemia, including nutritional and Addisonian anemias, with synthetic folic acid. In 5 of the 6 patients with leukopenia in addition to anemia, the white blood count rose under treatment. Pellagrous glossitis healed in 4 to 10 days, and burning of the tongue without objective glossitis cleared in the same interval. The onset of remission was accompanied by marked increase in appetite in most patients. In 2 cases with neuromuscular complaints, there was incomplete relief of paresthesia. The authors believed that the response was submaximal in that only 6 of 14 patients attained a hemoglobin level of 12 gm. % and 1 had a second reticulocyte response when treated with 50 U.S.P. units reticulogen after 18 days of *L. casei* factor, 20 mg. intramuscularly, daily. The longest period of treatment was 30 days and the dosage varied from 20 mg. daily parenterally to 150 mg. daily by mouth. Moore and his associates[21] treated 4 patients with macrocytic anemia, including 2 with Addisonian pernicious anemia, with folic acid. On the 3rd day of treatment with 100 mg. orally daily, subjective improvement was noted. The reticulocyte response began on the 4th day and reached a peak of 40% on the 7th day. Treatment was continued for 10 days, at which time the red blood count was slightly over 3 million. They concluded that this form of therapy was insufficient for a complete hematologic response. They treated 2 other patients with 2 mg. folic acid intravenously with what was interpreted as a submaximal response.

Spies[29] reported the successful treatment of a group of 26 cases of macrocytic anemia of various types with folic acid in doses of 10 mg. or more daily. Subjective improvement was noted on the 3rd day, and the reticulocyte response began on the 3rd to 6th day, reaching a peak of 6.4 to 31.8% on the 6th to 8th day. All elements in the peripheral blood tended to return to normal. He noted that on this therapy, the megaloblasts in the bone marrow decreased. Spies and his collaborators[30] concluded that folic acid was effective in the treatment of Addisonian pernicious anemia, nutritional macrocytic anemia, the macrocytic anemias of sprue, pellagra and pregnancy. However, the response to yeast and liver extract seemed to be out of proportion to their folic acid content. A number of patients who failed to respond to 3 to 4 mg. folic acid by mouth, responded to liver extract calculated to contain 1 mg. or less of folic acid daily.[29] The highest red blood count attained was 4.22 million with a hemoglobin level of 13.9 gm. per 100 cc. in a patient with an initial count of 2.01 million and hemoglobin level of 8.3 gm. per 100 cc., who received 100 mg. of folic acid by mouth daily for 40 days. Doan and his associates[13] treated 1 patient with pernicious anemia and combined sclerosis of the cord with 2 mg. folic acid parenterally for 15 days with results indistinguishable from those which would have been

expected with parenteral liver. No secondary reticulocyte response occurred when the dose was increased to 10 mg. daily. On the 10th day of therapy, the sternal marrow appeared normal. The maximum reticulocyte response of 26.8% was reached on the 15th day. On the 40th day of treatment, the red blood count was 4 million per c.mm. and the hemoglobin 13 gm. per 100 cc. (initial levels 1.49 million and 5.8 gm.). On the 2nd day of treatment, the patient's paresthesias disappeared and on the 21st day vibratory sensation and the Romberg test reverted to normal.

Zuelzer[35,36] stated that folic acid is a specific anti-anemic factor the lack of which results in megaloblastic dysplasia of the bone marrow elements and macrocytic anemia. He described an anemia of children, hematologically indistinguishable from pernicious anemia, although not constantly associated with achlorhydria, which responded to treatment with folic acid in doses of 5 to 20 mg. intramuscularly or 50 to 100 mg. orally daily for 1 to 3 weeks. When achlorhydria was noted before treatment, it cleared on this regimen. Both the bone marrow and peripheral blood returned to normal. The response to folic acid was indistinguishable from that to liver, and no second reticulocyte response could be obtained with one, after the other had been exhibited. Within 2 days of the inception of therapy, the bone marrow showed complete disappearance of the megaloblastic pattern. Children with other types of anemia had no response to folic acid.

It is interesting to note that a potent antipernicious anemia fraction of liver is effective neither in the treatment of vitamin M deficiency in monkeys[11] nor the vitamin Bc deficiency of chicks.[34] It has therefore been concluded that *L. casei* factor and the antipernicious anemia principle are different.[34] It may be more accurate to conclude that the antipernicious anemia principle is not a single factor, since folic acid does appear to be an effective antipernicious anemia factor. It may also be postulated that the erythrocyte maturation factor is folic acid or is synthesized by the human organism from folic acid. Other compounds with similar structure or conjugation compounds of folic acid may also be utilized in the synthesis of the erythrocyte maturation factor by the human organism, without having folic acid activity for other organisms. Thus, both the *L. casei* factor and the *S. lactis* factor,[2] the similarity of whose structure has been mentioned previously, are potent in promoting the growth of *S. lactis*, while only the former is potent for *L. casei*. The antipernicious anemia potency of the *S. lactis* factor remains to be determined.

Method. We have undertaken to study the therapeutic effect of synthetic folic acid* in the treatment of pernicious anemia. In addition, we attempted to determine the dosage which might be considered equivalent to a unit of liver (*i. e.*, the minimal dose which, when given daily by mouth, produces a maximal hematologic response). In the first group of patients (4 cases), we gave oral doses which in the experience of others had produced satisfactory effects. After we had established the efficacy of this therapy, the succeeding patients were treated with a dose that was expected to produce a submaximal response so that a second reticulocyte response might be tested for with increased doses[13] (3 patients).

Daily reticulocyte counts were performed by the method of drawing blood smears on slides previously coated with alcoholic brilliant cresyl blue. Hemoglobin determinations were made with a Sahli-Hellige hemoglobinometer in which 100% equals 17 gm. per 100 cc. Red cell, white cell and platelet counts were performed in the Neubauer counting chamber. The red cell volume was determined in the Wintrobe hematocrit on oxalated blood.

Case Histories. CASE 1. M. E., a 76 year old woman, complained of dyspnea, weakness, dizziness, numbness of her feet, precordial pain and palpitation of 2 weeks duration. She had become gray at the age of 30. She was told that she was anemic 25 years before, but no therapy was attempted. Physical examination revealed a well-developed and well-nourished pale old woman. The physical and neurologic exam-

ination was entirely negative except for pallor. Gastric analysis revealed achlorhydria, refractory to histamine. Gastrointestinal Roentgen ray series revealed no abnormality. Sternal marrow showed 3 to 4% megaloblasts on admission and 0.6% remained at about this level. The mean corpuscular volume was 125 cμ on admission and 99 cμ after 1 month of treatment. The platelet count was 130,000 per c.mm. on admission and 290,000 on the 13th day of treatment. Throughout her hospital stay,

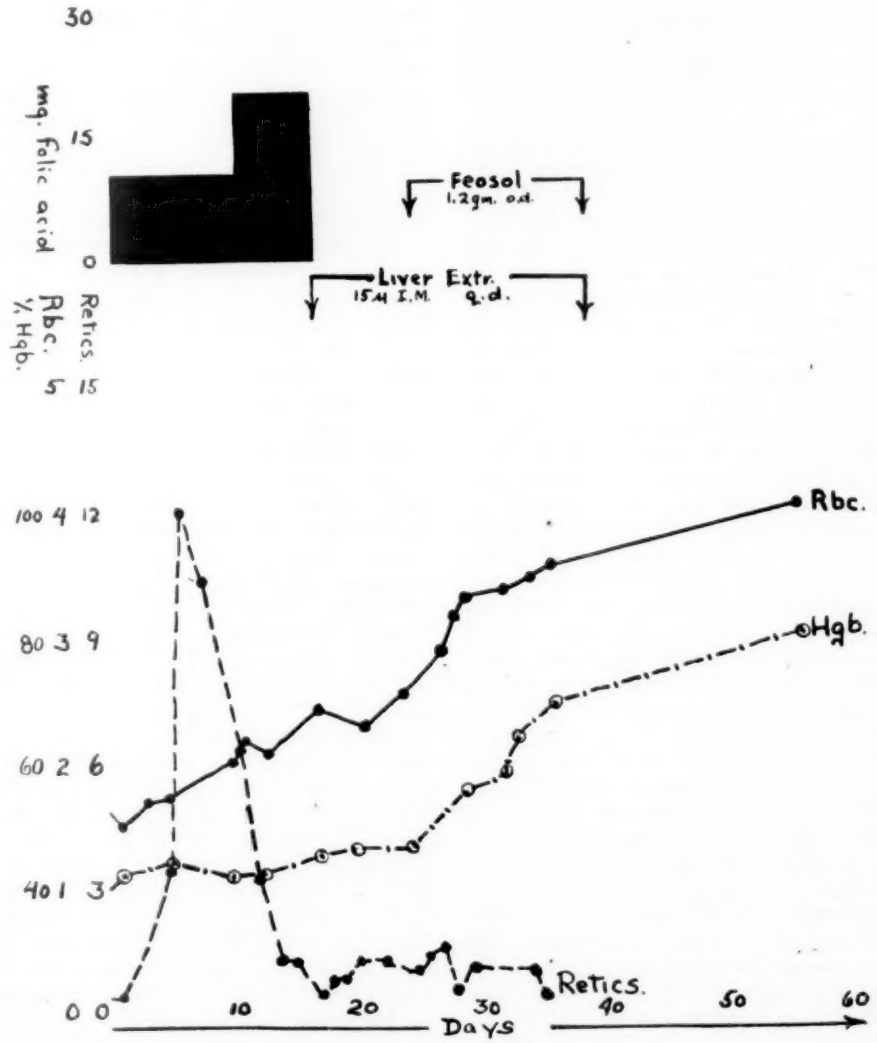

FIG. 1.—Hematologic response in Case 1.

megaloblasts after 2 weeks of therapy. The treatment with folic acid and later liver, and the hematologic response are described in Figure 1. Not shown is the white count, which rose from an initial level of 5000 per c.mm. to 11,000 while on folic acid, and she ran a low grade fever, always below 100° F. except between the 8th and 19th day when it was between 100° and 101.8° F. (peak on 12th and 13th days). There was only a slight upper respiratory infection to account for the fever. On the 8th and 9th

days of treatment, the nurse's notes revealed improved appetite.

CASE 2.—I. F., a 70 year old man, complained of fatigue for 1 year, slight icterus for 2 days. He had experienced an attack of gall bladder colic 4 years before admission, but no pain was associated with the present episode. The dietary history revealed poor protein and vitamin intake. Temperature on admission was 102° F. but subsided during the 1st week and remained normal thereafter. The liver was felt 2 finger-breadths below the costal margin and the spleen 1 finger-breadth. There was tenderness in the right upper quadrant. There was no glossitis and the neurologic examination was negative. Erythrocyte sedimentation rate was 76 mm. in 1 hour, icterus index 15, bilirubin 2.9 mg. % with delayed direct van den Bergh reaction. On the 3rd hospital day the icterus index was 9 and the bilirubin 1 mg. % respectively; and on the 5th hospital day the bilirubin was 0.5 mg. % and the direct van den Bergh negative. Alkaline phosphatase was 21 King-Armstrong units. The cephalin flocculation test was negative. Gall bladder Roentgen series revealed no filling of the gall bladder. Gastric analysis revealed no free acid after histamine both before and after completion of treatment. Gastro-intestinal Roentgen ray series revealed no abnormality. The bone marrow before treatment revealed the typical megaloblastic marrow (4%) of pernicious anemia. The hematologic response to folic acid therapy is shown in Figure 2. As noted, there was a reticulocyte crisis before the inception of folic acid treatment and a second response to the drug. This is discussed below. The white blood count rose from 2200 on admission to 5500 on the day specific treatment was begun, and remained at about this level. The platelet count was 290,000 before treatment and did not change significantly. The mean corpuscular volume was 110 $c\mu$ at the onset of therapy and fell to 95 at the time of discharge (7 weeks after admission).

CASE 3. C. P., a 64 year old Italian laborer, was admitted for the first time in September 1945 for epigastric burning, anorexia and weakness of 3 months duration. Gastro-intestinal series at that time was negative; gastric analysis revealed achlorhydria. Gastroscopy revealed marked atrophic gastritis. Bone marrow aspiration disclosed a typical megaloblastic marrow (5%). The hemoglobin was 59% and the red blood count 2.7 million. On yeast (60 gm. daily for 5 days) and liver extract (15 days), the hemoglobin rose to 78% and the red blood count to 3.66 million, but no reticulocyte response was noted.

Upon discharge in October 1945, the patient discontinued all therapy. He was readmitted March 1946 complaining of epigastric distress, anorexia, diarrhea, weakness and numbness of the fingers for 1 month. Examination revealed that the liver extended 3 finger-breadths below the costal margin. The spleen was felt 1 finger-breadth below the costal margin. There was moderate atrophic glossitis. Position sense was intact in the hands and feet but vibratory sense was diminished over the right tibia. Erythrocyte sedimentation rate was 30 mm. in 1 hour. Icterus index was 8, bilirubin 1.2 mg. % and the direct van den Bergh negative. There was gastric achlorhydria after histamine. Bone marrow aspiration again revealed a typically megaloblastic marrow. After 3 days of folic acid treatment the gastro-intestinal symptoms subsided and the patient noted marked improvement in appetite. Gastroscopy, performed after 15 days of therapy, revealed much less marked atrophy than was noted at the time of the first admission. Bone marrow aspiration performed 2 weeks after the onset of treatment revealed no megaloblasts, 59% normoblasts and 7% erythroblasts. Two weeks after admission, he noted disappearance of the complaint of numbness in his fingers. At the same time, the papillæ of his tongue began to regenerate, and the tongue became normal within 1 month. After treatment for 2½ months, the patient complained of stiffness and numbness of his hands, but there was no change in reflexes or proprioceptive sensation in upper or lower extremities. One month later, at which time his red blood count was 5.2 million and his weight was 151 pounds as compared to 140 on admission, he complained of increasing numbness in his hands and weakness in his knees. He stated that he had difficulty in buttoning his vest and tying his shoe-laces. There was suggestive minimal diminution in vibratory sensation at the right ankle, but no other change. He consulted another physician who treated him with liver extract and vitamin B complex intramuscularly. After the third injection, over a period of

2 weeks, he noted marked improvement in the numbness of his fingers, was again able to perform precise acts. When seen again 1 month after the induction of liver and vitamin B therapy, at which time he had received 6 injections, he had no complaints, but his neurologic findings, as before, showed slight impairment of vibratory sensation at the ankles.

The hematologic response to folic acid therapy, except for the white count, which rose from 4000 to 8000 (after 2 weeks treatment), is shown in Figure 3. The platelet count rose from 50,000 to 180,000 per c.mm. during treatment. The mean corpuscular volume was 158 $c\mu$ on admission, 110 $c\mu$ on the 14th day of treatment. It was not repeated again.

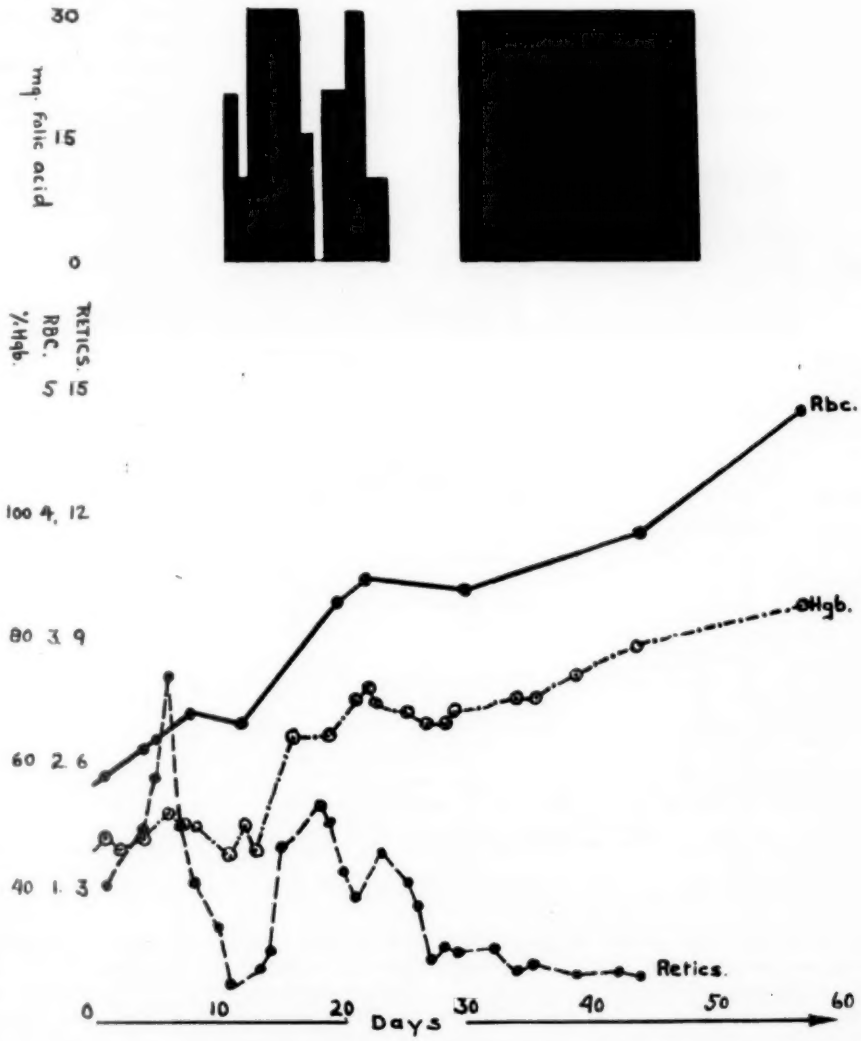

FIG. 2.—Hematologic response in Case 2.

CASE 4. O. S., a 67 year old Jewish woman, gave a 2 year history of epigastric and abdominal discomfort and weight loss. During the 2 months preceding admission her symptoms had become aggravated, associated with weakness, shortness of breath,

constipation and a loss of 10 pounds in weight. There was no glossitis. Liver and spleen were not palpable. Neurologic examination was negative except for slight diminution in vibratory sense at the knees and ankles. Erythrocyte sedimentation rate was 30 mm. in 1 hour. Gastric analysis revealed achlorhydria after histamine. The stools were persistently guaiac negative. Gastro-intestinal series and barium enema were negative. The bone marrow revealed 6.4% megaloblasts, 32% normoblasts, 0.8% erythroblasts. The peripheral blood was also typical of pernicious anemia. The mean corpuscular volume was 135 $c\mu$.

The patient noted marked improvement in appetite on the 5th day of therapy with 20 to 30 mg. folic acid daily, and her appetite became insatiable about the 7th day of treatment. Bone marrow aspiration on the 15th day of treatment revealed an active, essentially normal marrow, without megaloblasts. Platelets rose from 60,000 per c.mm. on admission to 230,000, 1 month later. White blood count was 3000 on admission, 5700 after 2 weeks treatment. Figure 4 describes the hematologic response. The only neurologic abnormality, diminished vibratory sensation in the legs, was slightly improved by treatment, in that after 2 months it became normal at the knees and was only slightly diminished at the ankles. Her weight increased from 108 pounds on admission to 116 in 2 months.

Case 5. M. F., a 71 year old Irish woman, had noted weakness, fatigue, anorexia and weight loss 5 years before admission for which she received sporadic injections of liver extract with slight improvement. She had received no treatment for 2 years, and for 6 months had noted the return of these symptoms associated with numbness of hands and feet so that walking became increasingly difficult. She had been bed-ridden for 1 month prior to admission.

On admission, the positive physical findings were marked pallor with a *cafe-au-lait* complexion, retinal hemorrhage and exudates, atrophic glossitis, sclerosis of peripheral pulses, impaired position and vibratory

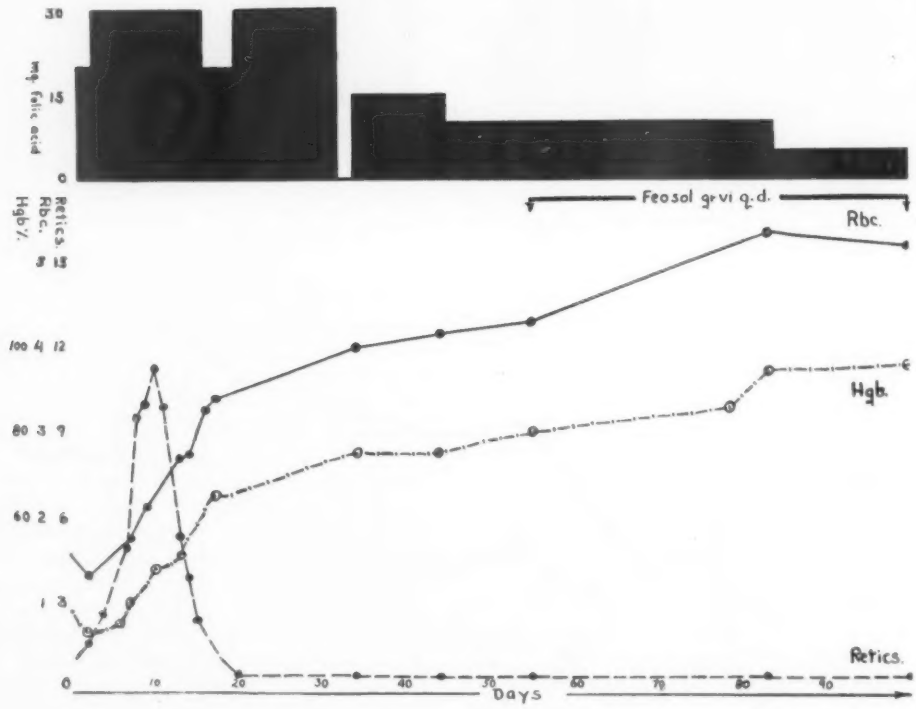

Fig. 3.—Hematologic response in Case 3.

sense in hands and feet, astereognosis, impaired pin-prick and light touch sensation in hands, active knee jerks (left more active than right), bilaterally positive Babinski's sign. Stool guaiac was negative, erythrocyte sedimentation rate 65 mm. in 1 hour. Gastric achlorhydria refractory to histamine was noted. Sternal marrow aspiration yielded an active megaloblastic marrow (16.8% megaloblasts).

By the 10th day of treatment, the patient stated that she was watching the clock for meal time and that the paresthesias in her fingers had improved. By the 22nd day, she stated that the paresthesias had disappeared entirely. The knee jerks were now equal and active and the Babinski reflexes had disappeared. Sensation in the upper extremities appeared to be normal. On the 30th day, she left her bed for the first time

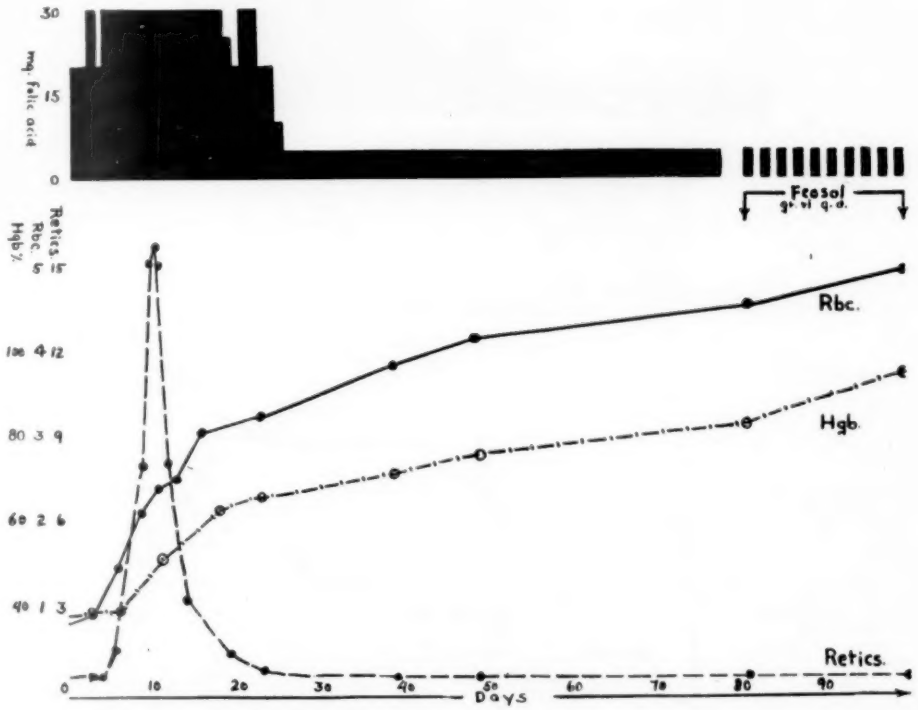

FIG. 4.—Hematologic response in Case 4.

She was treated with 5 mg. folic acid daily until the reticulocyte response had subsided, after which the dose was increased to 10 and then 30 mg. daily. On the 3rd day of therapy, improvement in appetite was noted. Bone marrow aspirated on the 4th day revealed 4% megaloblasts, which were considered atypical in that the nuclei stained more deeply, suggesting transition to erythroblasts. On the 6th day of treatment, new papillæ were noted on the tongue which began to feel rough to the touch. On the 8th day of treatment, only rare megaloblasts could be found in the marrow smears and on the 38th day there was none. and began to exercise in a walker. However, about 2 weeks later, she began to show signs of mental deterioration, complained of pains in her legs and refused to leave her bed. The objective neurologic findings remained static. She developed decubitus ulcers followed by a left lower lobe pneumonia with temperature up to 107° F. Urinary and fecal incontinence, which had been noted on admission and had improved during therapy, recurred. She died on the 4th day of the acute illness. Postmortem examination was not obtained.

Her hematologic response to physical therapy is shown in Figure 5. Not shown

are the rises in platelet count from 100,000 to 280,000 and in white count from 2000 to 6000 before the onset of pneumonia, when it rose to 10,000. The initial mean corpuscular volume was 118 $c\mu$.

insomnia. She complained of constant coldness of hands and feet, and noted considerable weight loss. For the past several months she had been treated with iron without effect.

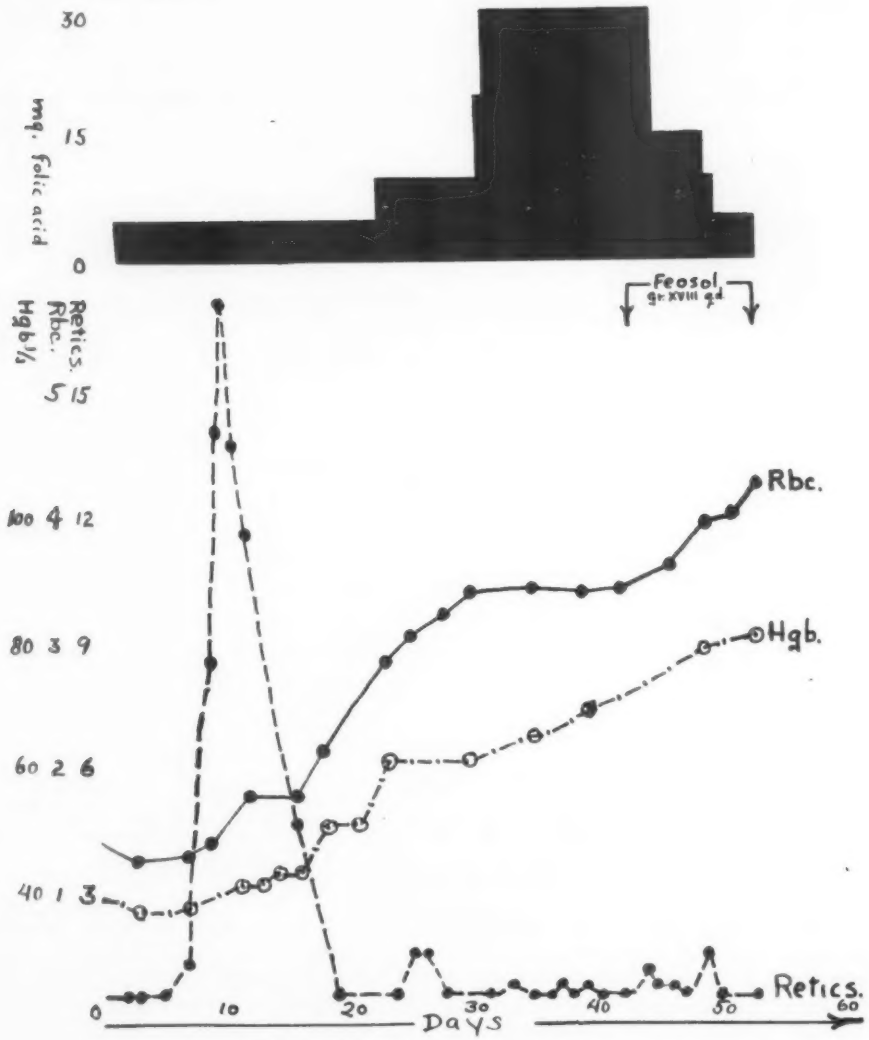

FIG. 5.—Hematologic response in Case 5.

CASE 6. P. M., a 59 year old Polish woman, had been in good health until 8 months before admission when she had an attack of gall bladder colic. The patient had always avoided fried and fatty foods, but restricted her diet more carefully following this episode. For the past 8 months she had noted anorexia, constipation and

Physical examination revealed lemon-yellow skin and scleræ. The fundi showed marked arteriolar narrowing and numerous petechial hemorrhages. The tongue was smooth and atrophic. Blood pressure was 110/64. There was marked sclerosis of the peripheral pulses. The deep tendon re-

flexes were equal and active. There was no impairment of vibratory or position sense.

The peripheral blood was typical of pernicious anemia: hemoglobin 32%, red blood count 1.2 million, white blood count 3500 with many hypermature neutrophils; there were marked poikilocytosis and anisocytosis, and a few megaloblasts and rare myelocytes were seen. Mean corpuscular volume was 120 cμ. Urinary urobilinogen was demonstrable in a dilution of 1:20. Stool guaiac was negative. Gastric achlorhydria refractory to histamine was noted before and after treatment. The bone marrow revealed 14.8% megaloblasts.

On the 8th day, new papillæ were noted in the central portion of the tongue. On the 16th day of treatment, she developed right upper quadrant pain with spasm and tenderness and fever to 103° F. These symptoms subsided after 7 days. A gall bladder Roentgen ray series failed to visualize the gall bladder, confirming the impression of acute cholecystitis. Following the subsidence of this episode, the patient again began to show subjective and objective improvement, followed by a second reticulocyte response to 2.5 mg. folic acid as shown in Figure 6. After this reticulocyte response manifested itself, she was permitted a regu-

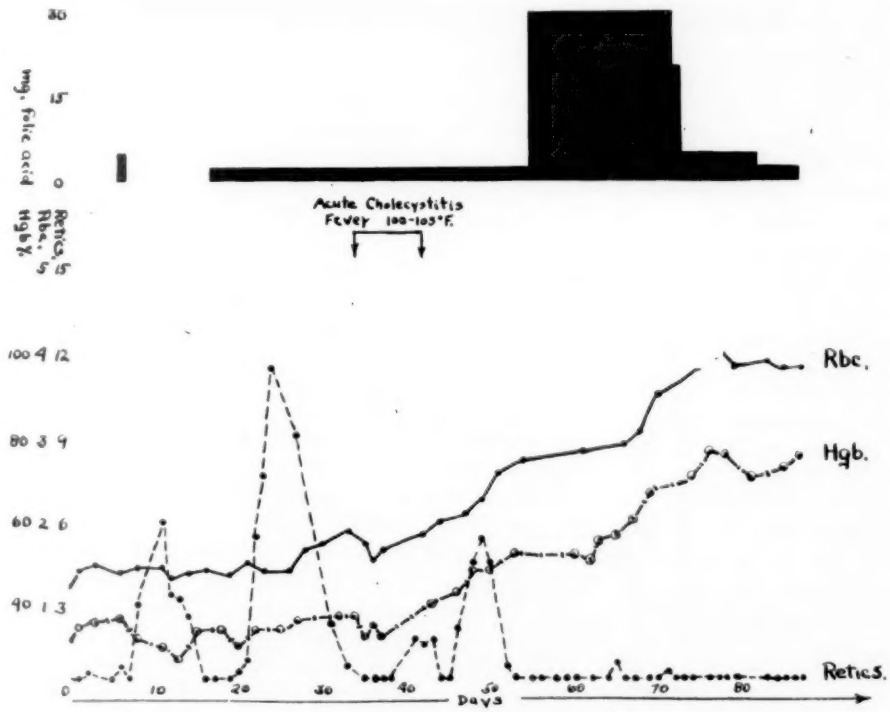

FIG. 6.—Hematologic response in Case 6.

The patient was placed on a diet restricted in sources of extrinsic factor and received a single dose of 5 mg. folic acid by mouth with reticulocyte response shown in Figure 6, but no subjective improvement. After the subsidence of this response, she was treated with 2.5 mg. folic acid daily. On the 4th day of this regimen she felt stronger, looked brighter, and stated that her appetite was considerably improved.

lar, low fat diet, without restriction of extrinsic factor When this response subsided, the dose of folic acid was increased to 30 mg. daily by mouth without secondary reticulocyte response (Fig. 6). The fundi and tongue appeared normal when reëxamined 2 months after admission. The neurologic examination remained normal throughout the period of observation. The white blood count rose from 3500 to 5000

(8300 during febrile episode) and the platelets from 100,000 to 240,000 in the 1st month. The mean corpuscular volume was 120 $c\mu$ on the 19th day of treatment with 2.5 mg. folic acid daily and 90 on the 40th day of continuous therapy.

negative. Physical examination revealed a pale, chronically ill male, appearing younger than his stated age. He was not dyspneic or orthopneic at rest. The fundi revealed arteriolar narrowing, arteriovenous crossing defects, numerous hemorrhages and exu-

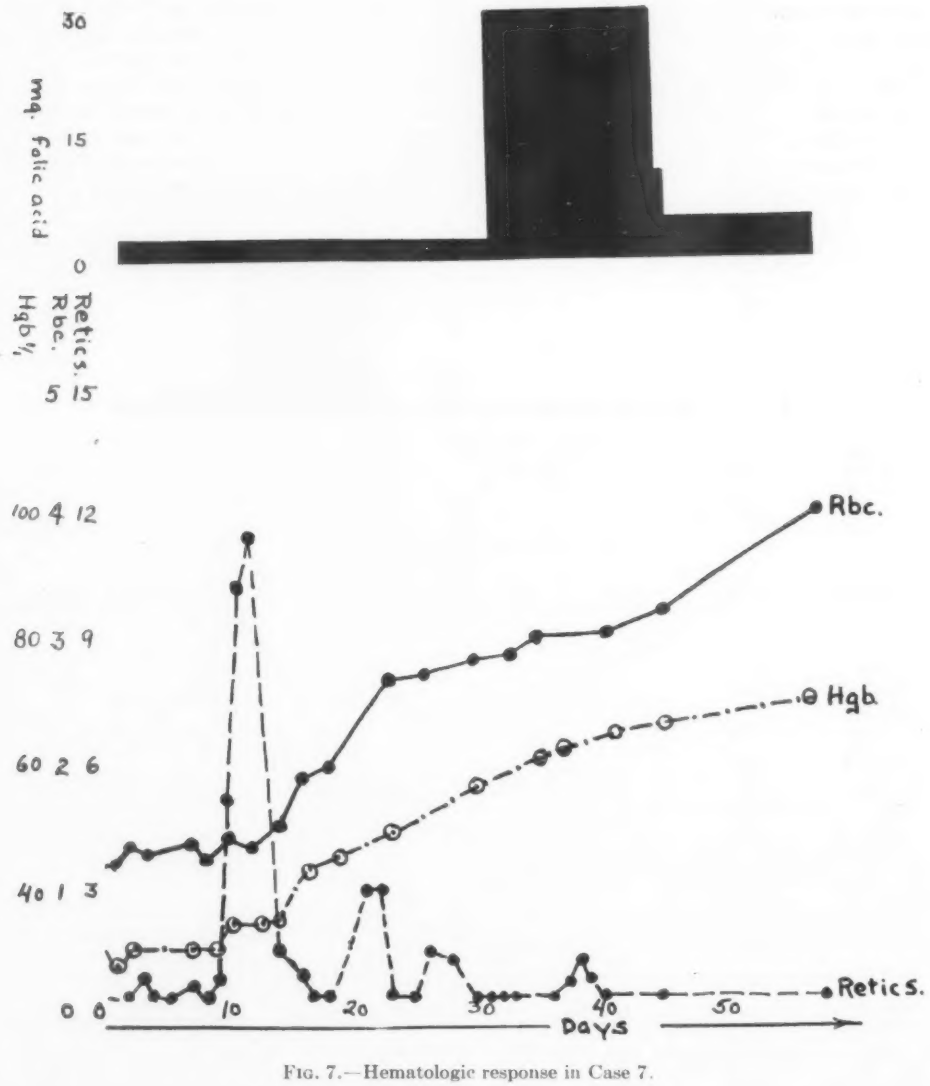

FIG. 7.—Hematologic response in Case 7.

CASE 7. J. M., a 70 year old Jewish man, complained of exertional dyspnea, sub, sternal oppression, orthopnea and anorexia of 6 months duration. Gastro-intestinal series, barium enema and chest plate made 3 weeks before admission were reported as

dates. There was no cardiac enlargement. A blowing systolic murmur was audible at the apex. The blood pressure was 125/60. The liver was palpable 1 finger-breadth below the costal margin. There was no glossitis or neurologic abnormality.

On admission, the peripheral blood smear revealed marked anisocytosis, poikilocytosis, 2 nucleated red cells per 100 white cells, reduced platelets (100,000 per c.mm.). The admission white blood count was 4400 with normal differential and many hypermature neutrophils. The bone marrow was typically megaloblastic. Gastric achlorhydria persisted after histamine. The icterus index was 12, bilirubin 1.4 mg. per 100 cc., direct van den Bergh negative and total protein 6.4 gm./100 cc. Urinary urobilinogen was positive in a dilution of 1:20.

The platelet count rose from 110,000 to 210,000 at the end of 2 weeks of treatment and the white blood count from 4450 to 7100. Treatment was begun with 2.5 mg. folic acid by mouth daily and increased to 30 mg. daily after subsidence of the reticulocyte response. The hematologic response is shown in Figure 7. On the 4th day of treatment, the patient noted increased strength, improved appetite and a sense of well-being. By the 55th day of treatment, he had gained 15 pounds.

In addition to the above 7 cases of pernicious anemia, 1 case of Hodgkin's disease with postradiation leukopenia, 1 ulcerative enterocolitis with hypochromic anemia, 1 aleukemic myelogenous leukemia, 2 patients with steatorrhea without anemia and 1 patient with regional ileitis were treated with 30 to 45 mg. folic acid by mouth daily without clinical or hematologic response.

Discussion. It is apparent from examination of the hematologic responses obtained (Figs. 1 to 7) that synthetic *L. casei* factor from liver is capable of producing a remission in patients with Addisonian pernicious anemia in relapse. In each case subjective improvement in appetite and general well-being was manifest about the 4th day of treatment. When gastrointestinal symptoms such as epigastric distress, anorexia or diarrhea were present, these subsided within a week. Glossitis disappeared in the same period, associated with beginning regeneration of papillæ. The reticulocyte response became manifest between the 3rd and 7th days and reached its peak between the 7th and 10th days. Leukopenia and thrombocytopenia disappeared. Achlorhydria was not modified. Neurologic complications, when present, were usually only minimally improved, but no progression was noted.

In Case 1, no secondary reticulocyte response was obtained to 15 units liver extract daily intramuscularly following 20 to 30 mg. folic acid daily by mouth. This indicated that the dosage of folic acid exhibited had produced a maximal hematologic response.[18] The hemoglobin and red cell rise was sluggish. The addition of iron resulted in a prompt response. This phenomenon was noted in other cases treated with folic acid, in which the hemoglobin became stabilized, usually at about 70% (11.9 gm./100 cc.) with a color index of 1 or less: upon the addition of iron to the regimen, the hemoglobin rose to normal values (Cases 3, 4 and 5).

In Case 2, there was a spontaneous reticulocyte crisis, probably related to the subsidence of the acute cholecystitis and the ingestion of an adequate diet. The patient's diet had previously been inadequate in protein foods. This response, however, was submaximal, since after its subsidence 20 to 30 mg. per day of folic acid produced a second reticulocyte response. The second response reached a peak of 5% reticulocytes on the 8th day of folic acid. The low peak can be ascribed to the fact that there had been a previous submaximal response of 8%.

Cases 3 and 4 illustrate the attainment of normal hemoglobin levels (over 85%—over 14.5 gm. per 100 cc.) with folic acid and iron over extended periods (2½ months of folic acid). The maintenance dose used in each case was 5 mg. orally daily.

In Case 6, a single oral dose of 5 mg. of folic acid produced a reticulocyte peak of 6% on the 6th day, which was not followed by a significant hemoglobin or red cell rise. After this reticulocyte response had subsided, 2.5 mg. folic acid daily produced a second reticulocyte response with a peak of 11.6% on the 8th day, indicating that the original single dose of 5 mg. had been inadequate. The red cell

and hemoglobin response was very sluggish. After the acute cholecystitis with fever up to 103° F. subsided, a further slight reticulocyte response occurred on the same dose of folic acid, without change in diet, and the hemoglobin and red cell count began to rise significantly. This would appear to indicate that in the face of the acute infection, 2.5 mg. of folic acid daily had produced a submaximal response, while the same therapy was adequate in the absence of intercurrent disease (as indicated by the lack of an additional reticulocyte response on an improved diet and increased folic acid dosage).

Cases 5 and 7 showed straightforward hematologic responses to 5 mg. and 2.5 mg. folic acid daily, respectively, with no further reticulocyte response to increased dosage.

It will be noted that the observed reticulocyte response fell short by about 50% of the expected response. However, this may be attributed to the method, since Minot and Castle's curves[18] are based on the determination of reticulocytes on smears made after the blood has been mixed with an aqueous solution of brilliant cresyl blue. The slide technique used in our laboratory gives considerably lower levels (*cf.* normal of less than 0.5% with normal of 1 to 1.5%). The erythrocyte response compares favorably with that obtained with liver.[14]

Conclusions. We may conclude that synthetic *L. casei* factor from liver is a potent antipernicious anemia factor, effective in producing a complete hematologic response, including reticulocyte count, red cell count, white cell count and platelet count, and correction of the megaloblastic dysplasia of the bone marrow. Reticulocytosis began between the 4th and 7th days and reached a peak 2 to 4 days later. As noted by other workers, subjective improvement, most strikingly evidenced by greatly improved appetite, occurred usually on the 3rd or 4th day of treatment. Glossitis and other gastro-intestinal symptoms subsided within 1 week, and regeneration of papillæ became apparent at this time. The minimal therapeutic dose, corresponding to one U. S. P. unit of liver is 2.5 mg. or less daily by mouth, since no second reticulocyte response could be produced in the 2 patients maintained on this dosage, by increasing the amount of folic acid. As with liver, larger doses are required in the face of infection. The failure of liver extract to produce a second response in 1 patient treated with 30 mg.

TABLE 1.—THE INITIAL RED COUNTS, CALCULATED EXPECTED RETICULOCYTE RESPONSE FOR ORAL LIVER AND NOTED RESPONSE

Patient	Initial R.B.C.	Calculated reticulocytes	Found reticulocytes
I. M. E.	1 6	20 4	12
II. I. F.	1 8	17 1	8, 5
III. C. P.	1 5	22 3	11
IV. O. S.	0 9	38 0	15
V. M. F.	1 4	24 3	17
VI. P. M.	1 3	26 5	6, 11.6
VII. J. M.	1 3	26 5	11 6

Signs of combined sclerosis of the cord were noted in 4 cases (Cases 1, 3, 4 and 5) and were severe in 1 (Case 5). In Cases 3 and 4, the disease was characterized by slight diminution of vibratory sense and paresthesias in the lower extremities, while in Case 1 there were paresthesias without objective changes. On treatment, these symptoms subsided. However, in Case 3 they returned after 2 months, while still on treatment with folic acid only to subside again with liver and vitamin B treatment. In Case 5 there was impairment of proprioceptive sense in the upper and lower extremities, and of motor power in the lower. The findings in the arms cleared under treatment, but those in the legs improved only minimally. There was no progression in the neurologic findings, but the patient developed a senile psychosis followed by a fatal bronchopneumonia.

of folic acid daily indicates that folic acid is capable of producing as complete a hematologic response as liver. By the addition of iron, the red cell count could be raised to normal levels on folic acid alone, given over a sufficiently long period (2 to 3 months). These results are at variance with those of Moore[21] and Vilter[32] and their co-workers, who failed to obtain complete remissions with folic acid. However, premature discontinuance of therapy may account for their inadequate response to folic acid. Combined sclerosis of the spinal cord was improved only very slightly by folic acid treatment. However, no case of development or progression of the neurologic disease during therapy was noted. In this respect, the experience is similar to that with liver. In the 1 case (Case 3) in which minor paresthesias recurred during treatment and again subsided on liver and vitamin B injections, evaluation is difficult because of the lack of objective changes and the confusion of the experiment by the administration of both vitamin B and liver extract.

Folic acid did not prove effective in the treatment of anemia and leukopenia due to acute myelogenous leukemia (aleukemic) or Hodgkin's disease, nor did it influence non-specific disease of the small bowel or colon.

It has been previously pointed out[21,29,34] that the antipernicious anemia potency of liver is out of proportion to its *L. casei* factor content and that liver fractions without *L. casei* potency are very active in erythrocyte maturation. The close chemical similarity, but microbiologic dissimilarity, between the *L. casei* factor (a compound of glutamic acid, para-aminobenzoic acid, and 2-amino-4-hydroxy-6-methyl-pteridine) and *S. lactis* factor (the same without glutamic acid), suggests that the human organism may be capable of utilizing a number of chemically similar compounds (with different microbiologic activities) in the synthesis of the erythrocyte maturation factor, or as the factor itself. This would account for the high antipernicious anemia potency despite low folic acid content (as measured by *L. casei* growth stimulation) of certain fractions.

Summary. 1. Seven patients with Addisonian pernicious anemia treated with folic acid are presented.

2. Adequate hematologic and clinical responses were obtained in each with doses varying from 2.5 to 30 mg. daily by mouth. The hematologic responses are shown graphically.

3. The minimal oral therapeutic dose for maximal response is 2.5 mg., or less, daily.

4. Combined sclerosis of the spinal cord is somewhat improved by folic acid therapy. The results are similar to those with liver.

5. It is suggested that the *L. casei* factor is either the antipernicious anemia factor or is utilized in its synthesis. Other compounds in liver with similar structures but without activity for *L. casei* may also be utilized by the human organism.

* The folic acid used in this study was supplied by Lederle Laboratories, Inc., through the courtesy of Dr. Stanton M. Hardy.

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RENAL FILTRATION RATES IN PREGNANCY TOXEMIA

INULIN AND EXOGENUS CREATININE

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ESTIMATES on rate of renal filtration, calculated from the excretory fate of injected inulin or mannitol,* depend upon a theoretically maximum no-threshold excretion of these sugars through kidney glomeruli, and upon an absence of their secretion or reabsorption in the tubules. According to Goldring *et al.*,[11] this figure amounts to 119 cc./min. plasma inulin in normal non-pregnant women. During uncomplicated pregnancy, filtration rates remain normal[12,20,21] but are reported by some[12,20] as slightly depressed and by others[8] as raised in the early puerperium.

Investigators record a considerable variation of filtration rates in those patients with pregnancy toxemia.[8] A tendency toward lower figures[6,8,12,18,20] (compared with normal pregnancy) and a comparative improvement postpartum[8,12,20] (contrasted with antenatal values) suggests interference with glomerular filtration. In most, the degree of antepartum suppression is commensurate with the severity of the disease.[12] Table 1 lists certain acceptable mean values for normally pregnant patients and for women with vascular syndromes, ante- and postpartum, and in subsequent follow-up.

Others[8,20] divide patients with toxemia into 3 groups as to kidney function. The first includes those with diminished inulin and normal (or elevated) diodrast clearances,† the filtration fractions,‡ by consequences, being lowered. This group belongs clinically to a preëclamptic type of pregnancy toxemia. Another includes those patients with normal inulin, decreased diodrast, elevated filtration fractions, and alleged evidence of essential hypertension. In a third group function tests are entirely normal. These observations are related to certain pathologic changes, a theoretically thickened glomerular basement membrane in the first group, and spasm, or organic change, in efferent renal vessels of the second. In the third group, that associated with normal function, the vascular status is considered relatively benign.

More recent studies[3,7,15] on eclampsia and severe preëclampsia record a comparative antepartum suppression of uric acid clearance. This reduction, observed during short and prolonged clearance intervals,[3,17] may relate to the blood elevation of this fraction in severe cases. Moreover, a mechanical interference with normal filtration, from a thickening of the glomerular basement membrane, might explain this increase in blood uric acid, although alterations in its tubular reabsorption occur in some severe toxemias. Bonsnes and Stander[3] have observed a few cases with a normal urea but suppressed uric acid clearance. The accompanying elevation of blood uric acid without retention of other metabolites is puzzling. Difference in rate of tubular reabsorption or secretion, instead of selec-

* Inulin and mannitol are non-metabolized polysaccharides of high molecular weight. The filtration rate is obtained from quantity of inulin or mannitol excreted per minute (UV) divided by their plasma concentrations (P). Filtration rate (C_I) = UV/P.

† Mammalian kidneys excrete injected diodrast or para-amino hippuric acid by tubules and glomerulus. Because these dyes are removed from arterial blood in a single renal circulation (rapidly excreted, largely by tubules) their clearances express renal blood flow at low plasma concentrations and tubular mass (TM) at high. The formula, $C_I = UV/P$, affords either calculation.

‡ The filtration fraction (FF), the apparent fraction of plasma cleared through glomeruli, is given directly by inulin/diodrast ratio.

tive rejection by a supposedly inert membrane, the glomerulus, would best explain this observation; providing, of course, the modern and accepted theory of renal function is correct.

The following data, previously reported in part,[7] and representing experience with simultaneous inulin, exogenous creatinine and urea clearance tests, are compared with contemporary results.

TABLE 1.—GLOMERULAR FILTRATION RATES—MEAN INULIN CLEARANCE VALUES
Expressed Cc./Min. Plasma (Adapted 3, 4)

	Normal pregnancy		Toxemia (cure*)		Toxemia (hypertension)		Follow-up toxemia ½ to 12 yrs.
	(3)	(4)	(3)	(4)	(3)	(4)	(3)
Antepartum	124	116	108	84	91	87	
Postpartum	116	139	120	105	110	95	94 3

* Taylor and co-authors[20] differentiate those patients followed by clinical cure from that group with subsequent hypertension.

Material and Method. Twelve pregnant women with vascular syndromes, 8 with signs of preëclampsia and 4 with hypertension, were selected for study. Of these, 5 were investigated ante- and postpartum. Additional studies were performed upon 2 normally pregnant patients, and upon 1 nonpregnant hypertensive woman.

Commencing at 0600,* after a previous midnight fast, the patient drank 200 cc. of water every ½ hour until completion of test. Breakfast consisting of 1 slice of unbuttered toast, and 200 cc. of milk instead of water, was consumed at 0730.* At 0800§ 10 gm. (100 cc.) U. S. Standard inulin was injected at the rate of 10 cc. (1 gm.) per minute, followed ½ hour later by 5 gm. creatinine by mouth. The clearance period, lasting 1 hour, commenced promptly at 0900.*

Blood specmens were obtained prior to 0800* for blanks and at exact mid-clearance period. Urine specimens were collected prior to 0800* for blanks and subsequently by catheter. Inulin analyses were as described by Alving *et al.*,[1,13] the Folin methods being used for exogenous creatinine and urea. The formula, Cm = UV/B, calculated inulin and exogenous creatinine clearances, whereas the usual 2 formulas of Van Slyke were used for urea. Clearance values were corrected to a surface area of 1.73 sq. m.

Results and Discussion. A total of 46 inulin, 26 exogenous creatinine, and 41 urea clearance determinations were obtained simultaneously from 15 patients. These results are tabulated (Tables 2 and 3). Patients were classified as to type of toxemia according to usual prerequisites, the final diagnosis being established in a subsequent 7 year follow-up.

1. URINARY OUTPUT. Employing a modified water diuresis test Dieckmann[7] observed a reduced antepartum urinary flow, expressed as % of intake, in patients with toxemia. In this series experimental conditions necessitated a controlled intake ante- and postpartum. Resultant figures, computed as % *increase* in output postpartum, are quite labile (551% mean average for 5 patients, or 371% for 12 patients with toxemia), but suggest an improvement in urinary flow following delivery. In general, ante- and postpartum urine volumes paralleled corresponding clearance figures for inulin and exogenous creatinine. It would appear that low renal outputs were directly concerned with the number and permeability of active glomeruli and a maximum constant rate of water reabsorption in tubules.

2. UREA CLEARANCE. According to established standards[7] the average values for preëclampsia and hypertension, ante- and postpartum, are 55 and 79% for specific toxemias (former) and 64 and 76% for the latter; whereas figures for normal pregnancy are 102 and 125%. In Table 3 results are converted to % normal; these agree quite well with above standards, 56 to 92% for preëclampsia and 81 and 77% for hypertension. In general, urea clearance values paralleled those of inulin and exogenous creatinine.

* Military time.

3. Exogenous Creatinine Clearance. Although clearance rates for endogenous creatinine approximate those for inulin or manitol,[14,19] it is contended that the former is excreted partly by tubules, particularly in subjects with depressed renal function.[14] Certainly the oral ingestion of creatinine materially raises its clearance.[14] This

Table 2.—Glomerular Filtration Rate—Inulin, Creatinine and Urea

No.	Diagnosis	Ante- or postpartum	Urine (cc./min.)	Plasma clearance (cc./min.) Inulin	Plasma clearance (cc./min.) Exogenous creatinine	Urea clearance rate
1	Hypertension	Ante	0.53	99	76	42.0
			1.13	86	..	32.5
		Post	5.53	141	179	74.0
			2.05	114	151	46.0
			7.00	159	..	67.0
2	Preëclampsia	Ante	1.60	130	..	17.0
		Post	3.66	233	..	96.0
			3.66	206	..	77.0
3	Preëclampsia	Ante	0.83	91	..	35.5
			0.46	74	..	35.0
		Post	3.46	118	98	64.0
			2.50	122	185	35.0
4	Hypertension	Post	2.90	88	68	55.0
			2.30	128	..	47.0
5	Preëclampsia	Ante	1.25	108	123	64.0
		Post	9.55	250	195	150.0
			8.20	219	275	121.0
6	Preëclampsia	Post	9.70	139	207	67.0
			7.10	148	201	63.0
			6.05	124	191	60.0
7	Preëclampsia	Ante	0.37	62	..	15.0
			1.07	46	137	25.0
			0.60	50	99	28.0
			0.93	64	132	29.0
			0.80	40	155	33.0
		Post	8.30	212	222	77.0
			7.50	111	191	55.0
			6.50	136	171	54.0
8	Hypertension	Ante	3.60	176	277	82.0
9	Preëclampsia	Post	6.50	105	..	53.0
			5.00	138	..	66.0
			3.80	165	..	57.0
10	Preëclampsia	Ante	2.93	138	219	67.0
11	Preëclampsia	Ante	1.63	67	..	20.0
			2.33	121	..	35.0
			2.37	102	..	34.0
12	Normal preg.	Ante	4.00	107		
			3.00	90		
13	Normal preg.	Ante	1.98	127	94	40.5
			1.26	130	115	44.0
14	Hypertension	N*	5.25	182	286	100.0
			3.80	133	215	67.0
			0.90	135	178	45.0
15	Hypertension	Post	1.10	135	..	30.0
			2.23	129	..	51.0
			5.90	134	..	48.0

* Not pregnant.

latter observation, interpreted as definite evidence of its tubular excretion, invalidates use of an exogenous method for measurement of filtration rates. In this series the exogenous creatinine clearance rate usually exceeded that for inulin.

4. Inulin Clearance. According to Dill and associates,[8] filtration rates for normal pregnancy are 116.14 ± 17.9 antepartum and 139.3 ± 16.1 postpartum. Thus, in this series, the mean inulin value antepartum was less than the lower limits of mean inulin deviation for normal pregnancy (Table 3). Antepartum values for preëclampsia are therefore comparatively low, improve markedly postpartum, and generally indicate interference with glomerular filtration in acute stages of the disease. In hypertension little change ante- and postpartum was manifest. A wide mean deviation occurred in toxemic patients. But, in general, these figures agree with those of previous reports (Table 1). Note, however, the tremendous diuresis manifested by some postpartum patients (Nos. 5, 6, 7 in Table 2). Urinary outputs of 540 to 600 cc./hour approach limits for maximum renal excretion. Glomerular filtration rates were high in some of these cases.

Table 3.—Glomerular Filtration Rate—Computed Averages

	Total		Plasma (cc./min.) Inulin	Creatinine	
	Patients	Clearances	Mean	Mean	Urea mean % normal
Preëclampsia:					
Ante	6	13	84	144	56
Post	6	15	161	193	92
Hypertension:					
Ante	2	3	120	176	81
Post	3	8	153	132	77
Normal pregnancy:					
Ante	2	4	96	104	
Hypertension:					
Non-pregnant	1	3	150	226	101

The low antepartum filtration rates, at least in specific toxemias, seems to be a consistent finding. But this reduction is scarcely sufficient to explain the markedly disturbed water and electrolyte balance present in such patients. The dramatic improvement in postpartum filtration rates constitutes the chief phenomenon demonstrated by this method, although its cause, hormonal or mechanical or otherwise, remains in doubt.

Employing an azocarmine stain, Bell[2] describes a thickened glomerular capillary membrane in kidneys from eclamptic patients. The comparatively diminished filtration rates, observed in specific toxemias, have been assigned to this lesion which supposedly interferes mechanically with the normal filtration process.[6] The consistent appearance of such a lesion in all cases of preëclampsia and eclampsia is questioned. Moreover, the rapid change from abnormal antepartum to normal postpartum filtration rates, covering so little time as 96 hours in this series, makes anatomic changes unlikely.

Studies must be obtained from women with severe preëclampsia, during the acute phase, if these specialized methods are to be more completely evaluated. Most patients in this series had symptoms of severe toxemia. But all of these did not have suppressed inulin rates. One woman, No. 2, with muscular twitching, mental stupor, epigastric pain, hypertension, 4+ albuminuria and headache, had a normal inulin clearance, 130 cc./min. On the other hand, a second patient, No. 1, in whom toxemic symptoms were less severe, exhibited the most marked antepartum inulin suppression, 40 to 64 cc./min. (Table 2).

The appearance of oliguria or anuria during pregnancy toxemia constitutes a serious prognostic sign. With the advent of newer methods for kidney research it was hoped the mechanism of this suppression would become explained. Such was not the case. Most published data have been collected from patients with adequate urinary outputs; and it was impossible to measure clearances by the usual methods in women with anuria. This last difficulty could be obviated by injecting diodrast or p-amino hippuric acid, introducing a venous catheter (*via.* jugular to vena cava) to renal vein levels, collecting that blood and brachial arterial blood, and calculating renal blood flow. Such an extended procedure, however, is not always advisable. The following case report illustrates the difficulties encountered in investigating renal function in patients with anuria.

Case Report. CASE 1. A. S. (No. 6317), an unregistered 24 year old septagravida, entered the hospital near term in coma with history of 3 convulsions. There had been a previous toxemia as well as hypertension during present gestation. In addition, a blood pressure of 230/160, 4+ albuminuria and normal fetal heart were noted. Usual therapy, consisting of hypertonic glucose, sedation and bag induction of labor, was followed by clinical improvement. However, 200 cc. sucrose was administered intravenously twice in the next several hours. Convulsions and coma reappeared. Anuria developed and the patient expired 24 hours after admission. Blood chemistry analyses disclosed depleted blood chlorides and acidosis. Arteriolar spasm was visualized during ophthalmoscopy. Microscopic examination of kidneys revealed marked swelling of nephron cells, avascular glomeruli and fatty degeneration of tubules.

This case, representing a patient with chronic vascular disease and eclampsia, was not subjected to the increased manipulations which a specialized renal investigation would have entailed. The risk was too great. Note that the intravenous administration of hypertonic glucose was followed by some clinical improvement, but that injected 50% sucrose, injudiciously employed, terminated in anuria, convulsions and death. The depleted blood chlorides, 512 mg. per 100 cc. prior to injection and 244 mg. 4 hours later, contributed to the disturbed osmotic relationship. It serves to illustrate the advantage of intravenous hypertonic glucose which suppresses blood chlorides less and yet produces twice the osmotic effect of sucrose solutions. Large avascular glomeruli, typical for eclampsia, do not explain the presence of anuria. Urinary suppression is probably functional, not organic, in origin. Possibly the "fatty degeneration," observed in tubules of such cases, represents an effort at secretion rather than a degenerative process.

Probably the best opportunity to study renal suppression is found in toxemic patients with complicating oliguria. Chesley[4] has noted a suppressed endogenous creatinine clearance in oliguria induced by water restriction, and suggests that urine volumes vary with filtration rates. The results in this series, so far as low antepartum volumes are concerned, confirm that work, although the output was not artificially reduced, or below the level for oliguria, 0.5 cc./min. However, clearance tests conducted on patients with low urine volumes are notoriously inaccurate.[5,19] It is often difficult to insure complete urine collection, and, at low outputs, the numerator of the clearance ratio (U/B) suffers disproportionate variations. Earle and Berliner[10] propose a simplified procedure in which renal blood flow and glomerular filtration are measured simultaneously without the collection of urine specimens. A constant intravenous infusion pump delivers a measured amount of solution of known concentration. Since inulin (or mannitol) and diodrast (or p-amino hippuric acid) are eliminated almost entirely by the kidneys, clearance rates are calculated by dividing the amount of these chemicals injected per minute by their plasma concentrations. Such a method allows prolonged observation of

patients with oliguria and reduces errors usually ascribed to an inaccurate collection of urine specimens.

In the following case report, mannitol and p-amino hippuric acid were employed to measure filtration rate and renal blood flow.

CASE 2. P. K. (No. 375466), a registered 33 year old gravida 4, observed from the 12th week of pregnancy with hypertension, was induced near term, and delivered of a 2830 gm. living female infant. On the 8th postpartum day, a severe headache, dizziness, vomiting and blood pressure of 270/160 suddenly appeared. This was followed by a 6 hour period of oliguria, the hourly output being 10, 10, 65, 25, 10 and 20 cc. Mannitol and p-amino hippuric acid clearances were obtained. These were calculated as 53 cc. plasma/minute for mannitol, half normal value, and 650 cc. plasma/minute p-amino hippuric acid, an adequate figure for renal blood flow. During this hypertensive episode marked arteriolar spasm was detected in scleral vessels by capillary microscopy. Subsequently urinary function improved in this patient.

Transient oliguria has been produced experimentally by prolonged immersion of a limb in an ice-water bath.[7] In addition, arteriolar constriction has been detected by ophthalmoscopy during an ice-water test.[16] These observations suggest that oliguria is due to a reduced rate of glomerular filtration. The angiospasm, observed in scleral vessels and representative of identical process in renal arterioles, may cause the urinary suppression observed in some patients with pregnancy toxemia.

Summary. Simultaneous inulin, exogenous creatinine, and urea clearance tests were obtained from 15 women, 12 of whom had severe signs of pregnancy toxemia. Urinary output, under identical conditions of fluid intake, improved markedly postpartum. Results for urea clearance compared favorably with established standards. With few exceptions figures for exogenous creatinine clearance exceeded those for inulin.

In severe preëclampsia, inulin, exogenous creatinine, and urea clearance rates were comparatively reduced antepartum, paralleled each other, and improved during the puerperium. Considerable variation in inulin clearance values exists in patients with pregnancy toxemia.

Severe cases, particularly those with oliguria, cannot be accurately investigated by the usual clearance methods employing collected urine specimens. Consequently, additional evidence is necessary to determine the exact mechanism of oliguria and anuria. The possible etiologic significance of angiospasm is postulated.

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PLASMA ANGIOTONASE CONCENTRATION IN NORMAL AND TOXEMIC PREGNANCIES*

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The renal pressor system is the term commonly employed to describe the biochemical reactions mediating acute hypertension of renal origin. As illustrated in Figure 1, angiotonin (hypertensin) is formed by the proteolytic action of renin upon a protein which exists in small quantities in normal plasma, and is destroyed by an enzyme, or mixture of enzymes, called angiotonase (hypertensinase). Theoretically, hypertension could result from an increased concentration of either renin or renin substrate in the blood, or from a deficiency of angiotonase, and each of these possibilities has been suggested as a cause of experimental renal hypertension. The physiology of eclamptic hypertension has been discussed previously,[10] and the work of Kellar and Sutherland[5] supports the same thesis that the elevation of blood pressure in this disease is of humorally mediated chemical origin. Dexter and Haynes[2] demonstrated renin in the blood of 1 woman with eclampsia and in 2 out of 7 women with preëclampsia. They also studied the angiotonase levels of the plasma in human subjects with various hypertensive disorders without finding any significant variations, but their series included only 1 pregnant subject.[4]

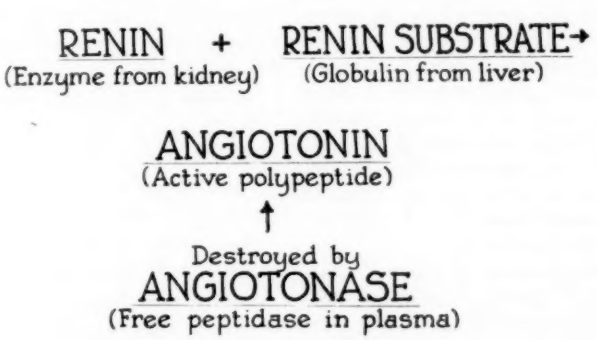

Fig. 1.—The renal pressor system

It is very likely that renin is present in small quantities in the plasma of all normal individuals,[8] and it is quite possible, in fact, that the renal pressor system is one of the homeostatic mechanisms for the maintenance of normal blood pressure.[7] If the angiotonase "deficiency theory"[3] accounted for the hypertension of eclampsia, one should find a consistent lowering of plasma angiotonase activity in this disease. The present study consists of a quantitative estimation of plasma angiotonase in both normal and toxemic pregnant women.

Methods. Renin substrate was prepared from beef blood by adding an equal volume of cold saturated solution of ammonium sulfate to 2 liters of serum, dialyzing the precipitate for 18 hours in the cold and redissolving it in 1% saline in an amount equivalent to one-tenth the original volume of serum. Angiotonin was prepared by incubating 200 cc. of the substrate solution with 0.5 gm. of commercial (Smaco) hog

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renin for 15 minutes at 40° C., then lowering the pH to 5.5 and precipitating the proteins in a boiling water bath. The filtrate containing the angiotonin was stored at 6° C. under toluol.

A smooth muscle preparation most sensitive to angiotonin is the rat uterus.[6] We found that the most satisfactory assays were obtained with the uterine horns of adult rats which have been ovariectomized about 2 weeks prior to use. This procedure abolished the spontaneous motility as well as the fluctuations of sensitivity due to the estrus cycle, but responsiveness of the uterus to angiotonin remained. In addition to its economy, the rat uterus method has the advantage of being insensitive to histamine, accurate to about the same degree as the blood pressure assay (plus or minus 20%), yet permitting multiple determinations on a small sample. The uterine horn is suspended in a 20 cc. capacity bath of Tyrode's solution maintained at 37° C. by a water jacket, and the writing lever is carefully counterbalanced for each strip. A stream of fine oxygen bubbles must come into contact with the uterus continuously. In matching responses, the mean rise of tonus, rather than the peak of the clonic response, is the more accurate indication of the dose. One "test dose" of angiotonin is the amount which produces an arbitrary 40 mm. response on the record (equivalent to an 8 mm. contraction of the uterine horn).

For the determination of plasma angiotonase, oxalated blood is collected with special precautions against hemolysis. Ten cc. of hemoglobin-free plasma is added to 40 cc. of 0.9% saline containing 50 "test doses" of angiotonin, adjusted if necessary to pH 7. An initial sample of 12 cc. is withdrawn immediately and inactivated with heat and acetic acid. The remainder of the mixture is incubated at 40° C., and 12 cc. samples are withdrawn at one-third less than the time estimated for half destruction, at the estimated half time, and at one-third more. (The time for destruction of half the angiotonin varies from 3 hours in a non-pregnant individual to about 30 minutes in a pregnant woman near term.) As each sample is removed, it is immediately inactivated and the filtrate is neutralized just before the assay. The percentage of angiotonin remaining in each sample may be plotted on the abscissa of semi-log paper, with the time in minutes on the ordinate. A straight line is best fitted to the 4 points, the initial sample always representing 100% angiotonin concentration at 0 time. In this way, the time for 50% destruction may be easily found.

Our unit of angiotonase activity is that described by Plentl and Page[13] and is based upon the kinetics of a first order reaction. Angiotonase units (a.u.)/cc. of plasma = $\frac{0.693}{\frac{1}{2} \text{ time (min.)}} \times 100 \times$ plasma dilution. In the method described, where the plasma dilution is always 1 in 5, a.u. at 40°/cc. = $\frac{346}{t\frac{1}{2} \text{ (min.)}}$

Results. The plasma angiotonase concentrations are illustrated in Figure 2. In 10 healthy non-pregnant subjects, there were from 0.9 to 2.5 units of enzyme activity/cc. of plasma, with a median value of 1.4. In the first half of pregnancy (5 cases), there was a moderate elevation, but this was not manifested during the first trimester. In the second half of pregnancy (11 cases), there was a progressive increase, the values ranging from 5.8 to 13.8, with a median value of 7.6 a.u./cc. Four samples of fetal cord blood showed the same concentration as the maternal blood (5.8 to 11 units). Plasma angiotonase falls to normal levels within a few days after delivery.

In 5 cases of preëclampsia, 3 gave normal values for the corresponding stage of pregnancy and 2 were low. Similarly, in 5 cases of eclampsia, 3 were normal and 2 were low. There was no correlation between the plasma concentration of angiotonase and either the severity of the disease or the level of blood pressure. The 2 most severe cases of eclampsia, for example, had the highest and the lowest values respectively, and the angiotonase concentration in the patient with the highest blood pressure (220/140) had a concentration of 10 a.u./cc. plasma.

Discussion. The cause of the 4 to 10-fold increase in plasma angiotonase in normal pregnancy is not known, nor is its physiologic significance clear. Other en-

zymes, such as histaminase[1] and pitocinase[9] show a similar though much more marked increase during pregnancy, and it is possible that both of these, together with the enzymes which we refer to as angiotonase, are contributed directly to the maternal blood by the placenta. Curiously enough, there are marked variations in the plasma concentration of all 3 enzymes in the presence of preëclampsia or eclampsia, supporting the conclusion that the enzyme changes are not the cause of the hypertension but the reflection of a more general and profound biochemical disturbance associated, perhaps, with an alteration of placental function.

angiotonin. This was tested on a short series of 9 patients by Dr. L. A. Sapirstein in the following manner: A slow intravenous infusion of normal saline was started and the blood pressure recorded every minute until a basal level was established. The angiotonin (0.4 cc. of a sterile solution kindly supplied to us by Dr. O. M. Helmer of the Eli Lilly Co.) was then introduced into the venoclysis tubing in order to avoid both the pain and psychic stimuli associated with venipuncture. The diastolic pressor response was plotted and the rate of disappearance was determined by extrapolation to the original base line. On 4 non-pregnant gynecologic patients,

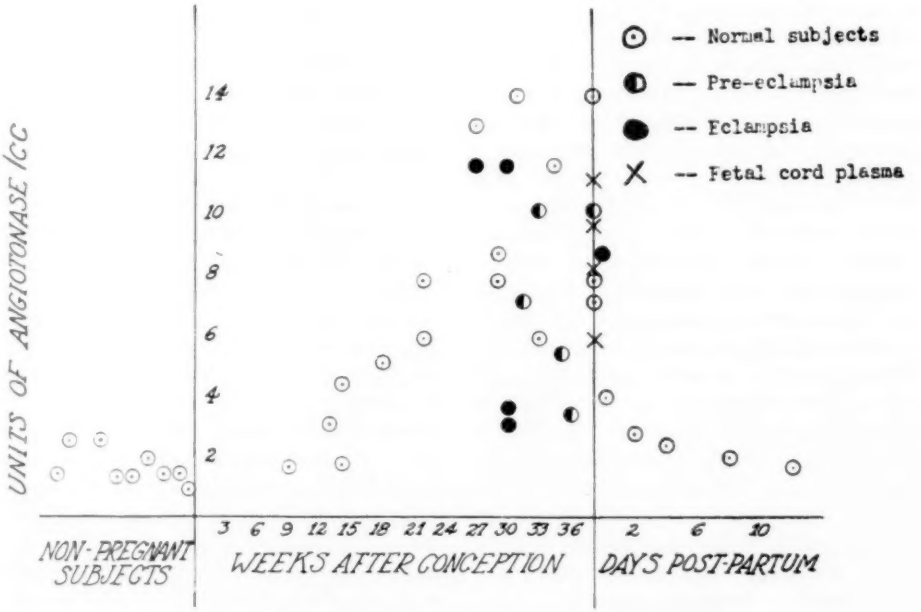

FIG. 2.—Plasma angiotonase concentrations during pregnancy

It was pointed out by Sapirstein, Reed and Page[14] that the greatest part, if not all, of the destruction of angiotonin under physiologic circumstances occurs in the plasma. The duration of the pressor response to intravenously injected angiotonin varies inversely with the plasma angiotonase concentration. One might anticipate, therefore, that pregnant women should show a diminished response to the average duration of blood pressure rise was 6¼ minutes (angiotonase level, 1.5 u./cc. plasma). On 2 puerperal women, 48 hours after delivery, the pressor response lasted 5½ minutes (average angiotonase concentration at this time is 2.8 u./cc. plasma). On 3 puerperal women 24 hours after delivery, the pressor response lasted only 3¼ minutes (average angiotonase value, 4 u. at this time).

While we did not test any women before delivery because of the possible risks of oxytocic effects of angiotonin, we would anticipate from these data that the blood pressure response of a woman at term to the same dose would last for only 90 seconds. By analogy, pregnant women would be far more resistant to the blood pressure raising effects of renin, inasmuch as the latter depends upon the formation of angiotonin *in vivo*, and therefore pregnant women would be less susceptible to hypertension resulting from renal ischemia. If the angiotonase content of the plasma is similarly elevated in the plasma of pregnant rats, it would account for the resistance of pregnant animals to the effects of renal ischemia[12] as well as for the decline of renal hypertension during the latter part of a rat's gestation, a decline which we have shown to be dependent upon the presence of a placenta rather than the fetuses or the endocrine changes.[11]

The fact that fetal cord plasma contains the same high levels of angiotonase as the maternal plasma suggests that this enzyme, unlike pitocinase, is able to traverse the fetal capillaries. We might anticipate that the enzyme would also be lost through the damaged glomerular capillaries in cases of eclampsia, along with other plasma proteins, and this would be the most likely explanation for the low plasma concentrations occasionally encountered in toxemic pregnancies with marked proteinuria. Unfortunately we have no data on the angiotonase activity of the urinary protein in these cases.

Summary. Assuming that the hypertension of eclampsia might be of renal origin, the possibility exists that it is based upon a deficiency of plasma angiotonase (hypertensinase) activity, since the primary site of angiotonin destruction is the plasma. Accordingly, the plasma concentrations of angiotonase were measured in 10 healthy non-pregnant subjects, 16 women with normal pregnancies, 5 cases of preëclampsia and 5 cases of eclampsia. Observations were also made upon the fall of plasma angiotonase after delivery, the concentration of the enzyme in fetal cord plasma, and the correlation between the plasma angiotonase level and the duration of the blood pressure response to intravenously injected angiotonin.

It was found that there is a 4 to 10-fold increase in plasma angiotonase concentration in the second half of normal pregnancy and a rapid return to normal after delivery. The same high levels were noted in fetal cord plasma. Puerperal women with elevated angiotonase values were found to have a diminished pressor response to intravenously injected angiotonin; it would be anticipated, therefore, that pregnant women (like pregnant rats) would be more resistant to hypertension resulting from renal ischemia.

In the cases of preëclampsia or eclampsia, 4 out of 10 cases had values of plasma angiotonase activity which were low in comparison to normal pregnancy, but still above the non-pregnant level. There was, however, no correlation between the degree of hypertension and the plasma angiotonase. This finding makes it unlikely that a deficiency of this "protective" enzyme or group of enzymes is the basis for eclamptic hypertension.

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DIAGNOSIS OF GENERALIZED AMYLOIDOSIS BY THE CONGO RED TEST: DEFINITIVE DIAGNOSTIC CRITERIA

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The problem of the diagnosis of amyloidosis has always been an important one in the management of certain chronic diseases, particularly tuberculosis. Widely recognized as a frequent complication in this disease, recent studies have shown it to be even more common than suspected. Auerbach and Stemmermann,[2] in a review of over 2000 consecutive autopsies at Sea View Hospital, show that it occurred in over 22% of cases coming to postmortem. Rosenblatt[17] found an incidence of 24.4% in 451 consecutive tuberculosis cases. Other authors have reported similar figures.[6,9,12] And even these statistics are probably underestimates, since they are based upon routine examinations without the regular use of special stains. If methyl violet stains were done in every autopsy, at least in the liver, spleen, kidney and adrenal, many more cases of minimal and moderate amyloidosis would be found. Be that as it may, tuberculosis is still one of our most common diseases and amyloidosis its most common complication.

Until the introduction of the Congo red test by Bennhold[4] in 1923, clinical diagnosis of amyloidosis was difficult and uncertain. Hepatosplenomegaly was the principal criterion and its frequent absence meant that diagnosis could be ventured with confidence only in advanced cases. Biopsy was of limited use since it was employed principally in those cases where the liver and spleen were palpably enlarged and anatomic confirmation was necessary, being used in this manner by Josefson[10] (spleen) and Waldenström[24] (liver). Therefore, the introduction of a simple biochemical diagnostic test was indeed a boon to the clinician.

The rationale of the test was based upon firm anatomic grounds. Bennhold showed (and he has since been amply confirmed) that amyloid substance has a special affinity for Congo red and when this colloidal dye is injected into the blood stream, it is removed from the plasma by the amyloid, so that when the blood is examined 1 hour after injection, the dye is no longer present ("absorbed"). Where amyloid is not present, most of the dye remains in the blood stream and is there found 1 hour later. Bennhold described 4 cases in his original paper who had complete absorption of the dye in 1 hour and who on postmortem showed amyloidosis. Soon after his paper appeared, other workers reported similar complete absorption confirmed by postmortem.[18]

However, difficulties with the test were apparent even during the first studies made with it. The first difficulty was that the test could not detect small amounts of amyloid. Bennhold described a case which had only 22% absorption and yet on postmortem showed splenic amyloidosis. Similar experiences of other workers led them to propose modification of the Bennhold technique to avoid this difficulty. Bennhold had injected 10 cc. of a 1% aqueous solution of Congo red and then had estimated the amount of dye present in the blood 4 minutes after injection ("the standard"). He next determined the amount remaining after 1 hour, the difference between the 2 specimens showing the amount absorbed during the intervening period. Paunz[15] used much smaller quantities of the dye in order to make the test more sensitive, and his technique has been employed by other workers, but his modifications, as well as similar variations,[5] have never gained wide popularity and Bennhold's method is still the basis for most of our present-day techniques.

Since technical improvement in the test have not been able to rid it of its inherent ability to detect small amounts of amyloid, this has become an accepted and tolerated deficiency, and is known as a "false negative" result.

A more important, and much more confusing, difficulty with the test is the "false positive." Here, the test indicates the presence of amyloidosis which is not there on postmortem. This has discredited the test with a number of workers. Jacobi and Grayzel[8] voiced this dissatisfaction in 1945 and state that because of this "The test must therefore be considered as of little moment in the absence of symptoms or signs of amyloidosis." However, this criticism of the test is a misdirected one. The trouble lies not with the test but with the interpretations with which it has been surrounded. This dates back to the original paper by Bennhold in which he states that absorption of over 40% is either amyloid or nephrosis and that absorption of over 60% is indicative of amyloidosis. But this statement was based on inadequate evidence which was not strengthened by those who uncritically accepted his criteria in the next decades. Auerbach and his co-workers have shown on the basis of extensive anatomic material that absorption of from 40 to 90% was found just as well in cases who did not have amyloid as in those who did.[11, 21] This question was recently thoroughly studied clinically and it was demonstrated that absorption of from 40 to 90% could under no circumstances be considered diagnostic of amyloid.[19] If this had been established during the early days of the use of the Congo red test, the number of so-called "false positive" tests would be far fewer and the test would enjoy greater popularity. This is not to say that cases showing less than 90% absorption may not have amyloid—such cases occur, but the test does not separate them from those who have no amyloid and who have the same percentage absorption. The test, in this absorption range, has no diagnostic value and we should not call upon it for aid which it cannot give.

Despite those cases of amyloidosis with incomplete absorption, in whom the Congo red test does not help us in diagnosis, the test is still of very great value. This is recognized by the wide acceptance of complete, or practically complete, absorption as diagnostic for amyloidosis. Schönberger and Rosenblatt,[18] 2 years after Bennhold's paper appeared, reported 3 cases which showed complete absorption of the dye; amyloidosis was confirmed at postmortem. Nathan,[14] although using smaller quantities of the dye, accepted as positive only those tests in which no dye is present in the 1 hour specimen. Moschkowitz[13] states that 100% absorption is reliable, and Cohen,[6] on the basis of his clinical experience, states that complete absorption is diagnostic of amyloidosis. Vivoli[23] accepted as proof of the presence of amyloidosis the removal of the dye from the plasma *en totalite* while Pearlman[16] considers absorption of over 90% of the dye as positive. Harmon and Kernwein[7] state their position, "If a positive result, by which we mean complete removal . . . is obtained, there is little likelihood that the condition is other than amyloid disease. Only 1 case[11] of a 'false positive' is on record if the criterion just mentioned is accepted." Finally, the work of these observers was reviewed, and their own extensive material was summarized, by Stemmermann and Auerbach[21] in 1944 who state that "90 or 100% absorption of Congo red dye is to be considered a positive result."

Nevertheless, despite these assurances, exceptions have been shown even to the rule that complete or almost complete absorption means the presence of amyloid substance in the body. This was first noted by Lipstein and Auerbach[11] in 1937 when they described 1 case of 100% absorption and 2 cases of 90 to 99% absorption which, on postmortem, showed no amyloid. Thirteen additional such cases were later reported by Stemmermann

and Auerbach,[21] this number representing 6.5% of Congo red tests done upon 200 patients in whom autopsy showed no amyloid present. Thus, it would seem that a false positive test is not an uncommon occurrence even when comparatively rigid criteria are used.

Recently, an extended clinical study was undertaken to see whether any criteria could be established which would avoid even the infrequent errors noted above. As a basis there was taken the widely accepted approval of 90 to 100% absorption as a positive test. A further lead was noted in Stemmermann and Auerbach's observation[21] that false positive tests, upon repetition, became negative.

Material and Methods. Sixty-one patients from the large patient population of Sea View Hospital were chosen for this study. Each case suffered from either pulmonary or osseous tuberculosis and had shown from 90 to 100% absorption on the Congo red test—this was the only criterion for inclusion in the group to be studied. Each patient was then thoroughly reviewed clinically, complete physical examination done, and serial Roentgenogramsand clinical course reviewed to establish the duration and course of the disease. Laboratory studies included multiple urine examinations for proteinuria, casts, red cells and specific gravity, with other tests done where necessary. From the above the clinical diagnosis of amyloidosis was attempted.

Each case was then retested with the Congo red test. The technique used was Taran's[22] modification of Bennhold's method, which has been adopted as standard by the Committee on Standard Laboratory Procedure of the American Trudeau Society.[1] This modification has been shown to result in fewer false negatives since colorimetric difficulties due to hemolysis are avoided.

Anatomic correlations were attempted in as many cases as possible, including those coming to postmortem and by biopsy in a much larger number. Biopsy was done of the liver or spleen in a few cases and of the gingiva in many others. The latter site has been shown to contain amyloid deposits in many cases of amyloidosis, although not in every case.[20]

Results. Our studies confirm the general proposition that complete or nearly complete absorption of Congo red can be used to establish the diagnosis of generalized amyloidosis. However, as will be noted below, several important conditions must be attached to this statement.

Tests Showing 90 to 99% Absorption. First of all, we have found that, contrary to previous statements, the absorption of from 90 to 99% of Congo red cannot be accepted as a reliable indication of the presence of amyloidosis. We studied 10 patients who showed 90 to 99% absorption. Upon retesting, 3 again showed 90 to 99%, while 7 on retesting showed less than 90% absorption. These figures are not as interesting or as informative as are the detailed analyses of the cases involved (see Table 1). Each of the 3 who showed no change on retesting had clinical evidence of amyloidosis; in addition, 1 had a positive gingival biopsy and another showed amyloidosis on autopsy. Of the 7 cases who had less than 90% on retest, 2 had clinical evidence of amyloid and each was proven morphologically, one by liver biopsy and theother by autopsy. The remaining 5 patients, however, showed no evidence of amyloidosis. Brief case reports follow:

Case 2. G. T. This patient is a 52 year old white male whose pumonary tuberculosis began in 1936, but has remained essentially non-progressive since that time and the patient is able to work at a part-time job. At no time has his liver or spleen been palpable and 27 examinations of his urine revealed a faint trace of protein 4 times. A routine test on Oct. 5, 1943, showed 90% absorption. The patient was retested on Feb. 4, 1946, but now showed 25% absorption. There is no explanation for this discrepancy.

Case 3. C. C. This patient is a 25-year old white female whose disease began in 1938. She was admitted to Sea View Hospital in 1939. A right pneumothorax controlled her disease on that side and a left thoracoplasty was performed in 1942, but her sputum continued alternately positive and negative. However, her clinical

TABLE 1.—DIAGNOSTIC STUDY OF 10 PATIENTS SHOWING 90 TO 99% ABSORPTION ON CONGO RED TEST

Case	Previous Congo red absorption (%)	Duration of disease (yrs.)	Course of disease	Clinical examination				Clinical diagnosis	Results of retest with Congo red (%)	Comment
				Liver	Spleen	Edema	Proteinuria			
1. R. B.	97	4	Quiesc.	1+	1+	2+	4+	Amyloid	85, 100	Gingival biopsy and autopsy both showed amyloid
2. G. T.	90	10	Quiesc.	0	0	0	0	Non-amyloid	25	
3. C. C.	90	7	Quiesc.	0	0	0	Occas.	Non-amyloid	50	
4. A. K.	98	6	Progr.	1+	0	0	2+	Amyloid	90, 100	Gingival biopsy positive for amyloid
5. J. L.	93	5	Progr.	2+	0	3+	4+	Amyloid	59, 64	Gingival biopsy and liver biopsy both showed amyloid
6. F. M.	90	9	Progr.	0	0	0	0	Non-amyloid	23	
7. H. P.	98	4	Quiesc.	1+	0	0	0	Non-amyloid	37	
8. R. L.	98	2	Progr.	0	0	0	1+	?	90	Gingival biopsy negative
9. O. D.	98	5	Progr.	2+	1+	3+	4+	Amyloid	90	Gingival biopsy and autopsy both showed amyloid
10. L. D.	95	2	Progr.	0	0	0	Occas.	Non-amyloid	29	Autopsy revealed no amyloid

TABLE 2.—SUMMARY OF DIAGNOSTIC STUDY OF 33 PATIENTS SHOWING 100% ABSORPTION ON CONGO RED TEST

Case	Previous Congo red absorption (%)	Result on retest	Comment
1–18	100	100	Gingival biopsy in each of these cases showed amyloid; autopsy was performed on 2 of these cases and in both showed amyloid
19	100	100	Gingival biopsy was negative; however, autopsy later showed amyloid
20–30	100	100	No biopsy was attempted in these cases
31	100	95	No biopsy was attempted; amyloid was present on postmortem
32	100	64	No clinical evidence of amyloid; see text
33	100	80, 29, 50	No clinical evidence of amyloid; see text

condition remained good and she was discharged in April 1946 for follow-up care. At no time during her hospitalization was there any clinical evidence of amyloidosis. Liver and spleen were not palpable and, except for occasional faint traces, her urine showed no proteinuria. On May 13, 1942, a Congo red test was routinely done and showed 60% absorption. Five months later another Congo red test showed 90% absorption. Retested once again on Nov. 10, 1945, she showed 50.4% absorption.

Summary. This patient never showed evidence of amyloidosis. There is no explanation available for the variation in the results of the Congo red test.

Case 6. F. M. This patient was a 27 year old white male whose illness began in 1937. A right thoracoplasty was done in 1939 and was revised in 1942. However, this therapy did not control the disease which was gradually progressive and the patient died on May 6, 1946, of pulmonary insufficiency. Three Congo red tests were done routinely. On Oct. 19, 1938, there was 60% absorption. On Mar. 7, 1941, there was 70% and on Nov. 26, 1945, there was 90% absorption. During all this time, the liver and spleen were never palpable and on many dozen urine examinations proteinuria was never found except on 1 occasion in 1941 during a febrile postoperative period. The patient was retested on Mar. 7, 1946, and was found to have 23% absorption. There is no explanation for the previous 90% absorption.

Case 7. H. P. This patient is a 40 year old colored male who was admitted to Sea View Hospital July 18, 1944, with a 2 year history of pulmonary tuberculosis. He stated that he habitually consumed large quantities of alcohol. His liver was slightly enlarged and firm on admission, but his spleen has never been palpable. Sixteen urine examinations were made—a very faint trace of albumin was present on 2 occasions. On August 13, 1945, a routine test was done preparatory to presentation to Surgical Conference and showed 98% absorption. This was repeated April 2, 1946, and then resulted in 37% absorption. There is no apparent explanation for this discrepancy.

Case 10. I. D. This patient was a 20 year old colored male who was found to have pulmonary tuberculosis during draft board examination in July 1944. He showed symptoms for the first time in March 1945 when caseopneumonic tuberculosis of the left lung was found. His disease ran an active course with spread to the right side and he died of a pulmonary hemorrhage on Mar. 6, 1946. At no time was his liver or spleen palpable. His urine shows occasional very faint traces of protein (during periods when his fever reached 105.8° F.). A Congo red test was done on Nov. 26, 1945, and showed 95% absorption. This was repeated on December 5 and only 29% absorption was found. Autopsy was performed and revealed caseopneumonic tuberculosis, disseminated miliary tuberculosis, intestinal and laryngeal tuberculosis. No amyloidosis was found.

Thus, it will be seen from the above that only 3 patients out of 10 who had from 90 to 99% absorption remained within this range upon retesting. Two others, who showed decreased absorption on retest, were clinically and morphologically proven amyloid. One-half of the patients showed negative results on retest with no evidence of amyloid and 1 of these was proven non-amyloid at autopsy. We, therefore, feel that absorption within this range is not diagnostic of amyloid.

Tests Resulting in 100% Absorption. Thirty-three cases were available for study who had had 100% absorption in previously performed Congo red tests. Clinical evaluation and Congo red retesting of this group leads to the conclusion that 100% Congo red absorption is almost, but not quite, diagnostic of amyloidosis. Retest of the patients in this group showed that 30 of them remained 100% on the second test. Gingival biopsies confirmed the diagnosis of amyloidosis in 18 of 19 in whom it was attempted—and the 19th later came to postmortem and showed marked renal amyloidosis. In 2 other cases of this group in which autopsy was done, amyloid was found. In 1 case, retest showed 95% absorption; amyloid was present on postmortem (Table 2).

The 2 remaining cases, nevertheless, were disquieting, both showing negative results on retest. Summaries of their case histories are presented below.

CASE 1. F. DeS. This patient was a 29 year old white female on admission to Sea View Hospital in April 1942, with a 6 year history of her disease. On admission, she was found to have pulmonary tuberculosis complicated by an empyema necessitatus. Thoracotomy, thoracoplasty and Schede were done in 1943 and resulted in conversion of her sputum and control of her disease. After revision of her Schede wound in 1945 she was discharged as arrested in January 1946. Clinically, no evi-evidence of amyloidosis was ever present except for occasional traces of proteinuria. A Congo red test done on Feb. 7, 1944, showed 25% absorption. In July, a second test showed 100% absorption and 1 done on Dec. 17, 1945, showed 64% absorption. The first test was done routinely before surgical conference, the second because of a palpable liver at the time of a biliary colic and the third in the course of this study. There was no clinical evidence of amyloid at any time. The reason for the 100% absorption is unknown—any relation to the liver involvement is conjectural. At the time of discharge the patient had no evidence of amyloidosis.

CASE 2. M. C. This patient was a 24 year old white female on admission to Sea View Hospital in 1941 with a 2 year history of pulmonary tuberculosis. A left thoracoplasty was done in 1942, converting her sputum, and she was discharged as arrested in 1943. She was re-admitted in August 1944 with a giant check-valve cavity in her right lung. In March 1945 a right cavernostomy was done and her sputum has been negative since. At no time while under observation has she presented any evidence of amyloidosis clinically. Five Congo red tests were done in this patient, the first 4 routinely before presentation to surgical conference and the 5th during this study. The results are listed: Nov. 27, 1941, 60%; Dec. 23, 1941, 100%; Jan. 14, 1943, 80%; Mar. 12, 1945, 29%; Feb. 11, 1946, 50%. There is no reason to believe that the 100% absorption represented amyloidosis. There is no explanation for the variation of these results.

Our experience in finding that 2 of the 33 cases who had had 100% absorption showed negative results on retest is by no means unusual, as we have noted above. This is quite in accord with the anatomic observations of Stemmermann and Auerbach[21] who noted that in 200 consecutive autopsies which showed no amyloid in any of the organs and in which Congo red tests had been done during life, 9 had shown 100% absorption being truly "false positives." It seems clear that a single 100% Congo red absorption cannot be considered as absolutely diagnostic of amyloid. Our findings also support the observation made by Stemmermann and Auerbach[21] that repetition of the test in patients with 100% Congo red absorption will tend to weed out cases without amyloidosis who might have shown, for some reason, complete absorption on their original test.

Tests Showing two Consecutive Complete or Nearly Complete Congo Red Absorptions. Since 90 to 99% absorption certainly shows no great diagnostic reliability and since even 100% absorption may occasionally be misleading, we have sought a possible definitive diagnostic criterion for the Congo red test. For this, we studied 21 patients who had shown, on 2 consecutive occasions, complete or nearly complete absorption of Congo red.

Our findings in this group were rewarding. Every patient who had shown a positive Congo red test on 2 occasions had a positive result upon retest (Table 3). Since this was in accord with our clinical and morphologic investigations of these patients, we have come to the conclusion that a definitive diagnostic criterion for the Congo red test may be established by requiring at least 2 complete or nearly complete absorptions before amyloidosis be definitely stated to be present.

These clinical impressions are in complete agreement with the autopsy experience at Sea View Hospital where there has never been a case seen which had 2 consecutive complete or nearly complete absorptions which did not have amyloid anatomically.[23] Every case which has shown 2 such absorptions has always shown amyloid.

TABLE 3.—DIAGNOSTIC STUDY OF 21 PATIENTS SHOWING 2 CONSECUTIVE CONGO RED TESTS WITH COMPLETE OR NEARLY COMPLETE ABSORPTION

Case	Previous Congo red absorption (%)	Duration of disease (yrs.)	Course of disease	Clinical examination				Clinical diagnosis	Result of Congo red retest (%)	Comment
				Liver	Spleen	Edema	Protein-uria			
1. A. R.	100, 100	15	Quiesc.	0	0	0	4+	Amyloidosis	100, 100 95	Gingival biopsy: positive for amyloid
2. A. K.	98, 90	6	Progr.	1+	0	0	2+	Amyloidosis	100, 100	Gingival biopsy: positive for amyloid
3. R. E.	100, 100	7	Quiesc.	0	2+	0	2+	Amyloidosis	100	Gingival biopsy: positive for amyloid
4. M. K.	100, 100	4	Progr.	2+	0	1+	4+	Amyloidosis	95, 100	
5. J. P.	100, 100	25	Quiesc.	3+	1+	0	1+	Amyloidosis	100	
6. E. C.	100, 100	12	Progr.	1+	0	0	4+	Amyloidosis	100	Gingival biopsy: positive for amyloid
7. H. H.	100, 100	15	Progr.	4+	1	0	4	Amyloidosis	100, 100	
8. H. K.	100, 100	7	Progr.	0	2+	0	1+	Amyloidosis	100	Gingival biopsy: normal gingiva
9. E. K.	100, 100	15	Quiesc.	2+	0	0	4+	Amyloidosis	100	Gingival biopsy: positive for amyloid
10. J. C.	100, 100	5	Progr.	2+	3+	0	4+	Amyloidosis	100	Gingival biopsy: positive for amyloid
11. S. S.	100, 100	10	Progr.	4+	3+	0	1+	Amyloidosis	100	Biopsy of spleen; pos. for amyloid
12. J. H.	100, 100	2	Progr.	3+	1+	0	3+	Amyloidosis	100	Gingival biopsy; positive for amyloid
13. H. Y.	100, 100	3	Progr.	3+	1+	1+	4+	Amyloidosis	100	
14. E. L.	100, 100	10	Quiesc.	1+	1+	0	2+	Amyloidosis	100, 98, 100 100, 100	Gingival biopsy: positive for amyloid
15. R. B.	100, 100	5	Progr.	4+	4+	0	1+	Amyloidosis	100	Gingival biopsy: positive for amyloid
16. R. G.	100, 100	12	Progr.	0	0	0	4+	Amyloidosis	100	Autopsy showed amyloid; gingival biopsy: normal gingiva
17. P. O'D.	100, 100	5	Quiesc.	2+	1+	0	1+	Amyloidosis	100	Gingival biopsy: positive for amyloid
18. L. C.	100, 100	5	Progr.	2+	0	0	4+	Amyloidosis	100	Gingival biopsy: positive for amyloid
19. R. L.	98, 98	2	Progr.	0	0	0	1+	Uncertain	100	Gingival biopsy: normal gingiva
20. C. S.	100, 100	2	Progr.	3+	1+	0	1+	Amyloidosis	100	Gingival biopsy; normal gingiva
21. J. D.	100, 100	5	Progr.	3+	0	0	1+	Amyloidosis	100	Gingival biopsy: normal gingiva

Discussion. There has now been accumulated sufficient clinical, experimental and anatomic data to assay reëvaluation of the Bennhold Congo red test and to define adequate criteria for its use. First of all, there is little doubt that it is a valuable weapon in our diagnostic armamentarium. There are few diseases in which so simple and so useful a test is available. It is to be regretted that the test is still insufficiently employed in many institutions.

We propose the following criteria for the evaluation of the Congo red test. These are based on the morphologic data of Auerbach and his co-workers and upon the clinical studies reported here and elsewhere.

1. *Those Tests Showing From 0 to 89% Absorption.* These results must be considered as negative. Patients without amyloidosis almost always fall within this group. While it is true that their results are usually in the lower half of this range, they may be scattered anywhere within it. An analysis of 1000 consecutive Congo red tests at Sea View Hospital reveals that 86.8% of all tests are in this group (Table 4).

TABLE 4.—RESULTS OF 1000 CONSECUTIVE CONGO RED TESTS

% Congo red absorbed	No. tests	%	
0– 9	64	6.4	
10–19	184	18.4	
20–29	202	20.2	
30–39	159	15.9	60.9
40–49	93	9.3	
50–59	64	6.4	
60–69	39	3.9	
70–79	30	3.0	
80–89	33	3.3	25.9
90–99	36	3.6	
100	96	9.6	13.2
	1000	100.0	100.0

Undoubtedly, there will be cases with amyloidosis which will show results within this range. In fact, out of 105 autopsied cases which showed amyloid, where the Congo red test had been done within 2 months of death, 24.3% showed absorption of less than 90%.[21] Thus, false negatives will be present, but we know of no way whereby the Congo red test, unaided by biopsy, can be used to differentiate them from cases without amyloidosis similarly falling within this absorption range. Nevertheless, these cases are not numerous and will not work too great a hardship on our diagnostic resources. They will occur, moreover, mainly in cases of minimal amyloidosis, which usually are of lesser clinical significance. This is an unavoidable deficiency of the test which must be put up with. Occasional false negatives must be expected if we are to avoid the greater embarrassment of the false positive.

2. *Cases Showing From 90 to 99% Absorption.* Patients who show this amount of absorption of injected Congo red often have amyloidosis. They have it more frequently than patients with, say, 50% absorption. But they frequently do not have amyloidosis, as has been seen from our studies above. Thus, unless clinical evidence of amyloid is present it would be unsafe to use the Congo red test with this amount of absorption as diagnostic evidence of amyloidosis. Therefore, absorption of from 90 to 99% is merely suggestive of amyloidosis (perhaps strongly suggestive when clinical evidence is present) but otherwise is of little aid.

3. *Tests Showing 100% Absorption of Congo Red.* A patient who shows complete absorption of Congo red 1 hour after the injection probably has amyloidosis whether or not clinical evidence is present. However, this is not always true. Occasional cases will occur when a 100% Congo red absorption will mislead us and will point to the diagnosis of amyloidosis when there is none present. We suggest that when 100% absorption is found a provisional diagnosis of amyloidosis be made but that it not be considered definitely established until the test be repeated and another complete or nearly complete absorption result.

4. *Tests Showing Complete or Nearly Complete Absorption on at Least 2 Occasions.* We believe that this rigid criterion should be established for the definitive

diagnosis of amyloidosis by the Congo red test: that a case not be considered amyloid unless 2 consecutive complete or nearly complete absorptions be obtained. We have never seen such a patient show a negative result on any further retest and no such patient has come to post-mortem without showing amyloidosis.

If the above criteria are adhered to, the Bennhold Congo red test will prove to be an exact instrument in the diagnosis of amyloidosis.

Summary. 1. The Congo red test is a valuable aid in the diagnosis of amyloidosis. It is based on the removal of intravenously injected Congo red from the blood stream by amyloid substance, if this be present.

2. Where only small deposits of amyloid substance are present, there will be failure to absorb appreciable amounts of Congo red. This will lead to a "false negative" result.

3. These patients, with small amounts of amyloid, show 0 to 89% absorption. Patients without amyloidosis give similar results. The test does not differentiate between them, and in this range has no diagnostic value.

4. There is widespread acceptance of 90 to 100% absorption as diagnostic of generalized amyloidosis. However, it has been shown that cases occasionally occur which show 90 or 100% absorption and yet have no amyloid. These are "false positive" tests.

5. Clinical studies were undertaken to determine definitive criteria for the diagnosis of amyloidosis by the Congo red test which would avoid even the infrequent "false positive." The conclusion was reached that a patient should not be considered amyloid unless there is complete or nearly complete absorption on 2 consecutive Congo red tests. We have never seen such a patient show a negative result on further retest and no such patient has yet come to postmortem without showing amyloidosis.

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PROGRESS
OF
MEDICAL SCIENCE

PEDIATRICS

UNDER THE CHARGE OF

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SICKLE CELL ANEMIA

RECENT PROGRESS OF PEDIATRIC INTEREST

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BECAUSE of its high rate of occurrence in the American Negro—approximately 7% by most estimates[5,9,14,26]—the sickle cell phenomenon has been subjected to hundreds of clinical and laboratory investigations. The variegated patterns of the disease picture have been portrayed comprehensively in the recent reports and reviews by Wintrobe,[31b] Lewis,[15] Mosely,[16] Murphy and Shapiro,[17a,b] and others. Agreement is universal that the disturbances exhibited by afflicted individuals can be attributed largely and probably entirely to some congenital and inherited defect of the red cell itself. The curious shapes in sickle cell anemia are expressions of the abnormality of the stroma. This survey summarizes some of the more recent advances in the deciphering of the biochemical mechanisms responsible for the sickling, and their relationships to the pathogenesis of symptoms and signs in childhood.

THE RED CELL. In 1939, Diggs and Bibb[6] reported on what was then known concerning the red cell in sickle cell anemia, based on review of the literature and personal observations of 47 patients with active sickle cell anemia at the John Gaston Hospital, Memphis, Tennessee. They pointed out that the structural alterations during sickling take place in a variety of ways, and that sickled cells take on a diversity of shapes and contours. Reticulocytes do not sickle as readily nor as bizarrely as do the more mature cells. Normoblasts undergo changes in shape even less readily, though they too may sickle. The type of cell adjudged most characteristic is "the hyperchromatic elongated form, pointed at each end and curved in the middle. . . . There is no sharp line of demarcation between the round, the oval, the blunt elliptic, the elongated elliptic, the elongated with one end and with two points and the curved cells with double points." Target cells may also be seen. The elliptic and oat-shaped cells are deemed to have become irreparably changed in shape.

The red cells may exhibit striking anisochromia, with diffuse basophilia, Howell-Jolly bodies, etc. Price-Jones curves of 10 patients revealed marked anisocytosis,

with average diameter greater than normal. Nucleated rell cells are common. The reticulocyte count is elevated, the average for 42 patients being 15%. More detailed studies of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration led Diggs and Bibb to concur with Wintrobe[31] that the red cells in sickle cell anemia are usually of the normocytic normochromic type, with a tendency toward macrocytosis. In the fragility test bloods from patients with sicklemia as well as sickle cell anemia displayed an increased resistance to hypotonic saline solutions. Some of the red cells of a few patients failed to break up even when suspended in distilled water. This increased resistance is ascribed to the ability of these cells to alter in shape without placing their membranes under sufficient tension to cause hemolysis.

The morphodynamics of the change in shape which takes place during sickling has been carefully observed by Ponder.[20] Those red cells which become sickle cells when the O_2 tension becomes reduced, he termed meniscocytes. In their usual discoid shape meniscocytes are indistinguishable from normal red cells and are referred to as premeniscocytes.

By pressing on the cover-slip in sealed wet preparations with a dissecting needle, Ponder was able, with the microscope, to observe meniscocytes in a variety of positions. Manipulation of position brought out a change in rigidity of the promeniscocytes before any recognizable change in shape. If a cell is turned up on edge, for example, the upper part tends to fall over in the middle like a sheet of paper, as if the cell were unable to sustain its own weight. A normal red cell, in contrast, has sufficient intrinsic rigidity to stand on end like a bit of cardboard. When currents in the fluid bring premeniscocytes into collision with one another one cell may bend almost at right angles in flowing over a rounded projection on another.

After the stage of change in rigidity comes the sickling phenomenon proper. The rim of the cell becomes thicker on one side and thinner on the opposite. The thinning proceeds until continuity breaks down. The 2 newly formed ends then become separated by straightening out of the arc, which flattens the biconcavity between them as the degree of curvature decreases. The thinned and stretched material of the biconcavity then expands at its unsupported edge into a series of veil-like projections. As the arc continues to straighten the veil-like material of the biconcavity follows the change of form of the arc, and in the completed meniscocyte appears as a very thin and almost invisible fringe lying within the sickle. The entire process of meniscocyte formation takes place usually within 1 and 2 minutes. Measurements show that in sickling the average cell increases in mean thickness but the volume remains unchanged.

When oxygen is reintroduced these processes reverse themselves, resulting in a normal appearing cell shape without any sign of where the biconcavity had been except for a small refractile globule which may not persist for very long.

To account for these shape changes, Ponder hypothesizes that the hemoglobin in the cell exerts an "expansive force." This force is a function of the oxygen tension which, in the normal red cell, can produce only tension or turgor because the surface ultrastructure is intact but which, in the premeniscocyte and meniscocyte, produces sickling because the surface ultrastructure is defective.

The essential mechanism for the development of the expansive force seems related to the conversion of hemoglobin from the combined to the reduced state. In cells which have been lysed by water the residual cell bodies no longer alter in shape when the oxygen tension of the suspending medium is changed. In the normal red cell the forces of surface tension tend to induce a spherical form, whereas the intermolecular forces of the surface ultrastructure tend to maintain the biconcave disk shape. If the ultrastructure is weak, as is assumed to occur in the

promeniscocyte, the form forces tend first to break, and then to distort, the C-shaped rim of the cell into the shapes which the meniscocyte successively assumes. Conversely, diminution of the turgor-producing forces by oxygenation of the hemoglobin allows the intermolecular arrangements in the surface ultrastructure to re-establish themselves, and the disk form becomes restored, essentially by progressive re-curvature of the C-shaped rim.

In 2 patients with sickle cell anemia Singer[23] observed a markedly increased resistance to lysolecithin. (Lysolecithin is a hemolytic substance contained in normal blood serum, which can be extracted and used as a means of testing red cell fragility.) A third patient, however, presented a normal lysolecithin fragility though the cells were saline resistant. Thus there was a lack of complete parallelism between the resistance of the red cells in sickle cell anemia towards hypotonic saline solutions and towards lysolecithin.

Studies on the blood of normal Negroes by Helm and Jacobs[12] revealed a high osmotic resistance of at least some of the red cells. In one woman with sickle cell anemia this peculiarity was greatly exaggerated.

Tomlinson and Jacob[28] concluded from experiments with washed red cells that carbonic anhydrase is probably present in normal active amounts in the red cells of persons with sickling, and that it does not enter into the phenomenon of sickling beyond the normal function of facilitating the exchange of carbon dioxide and oxygen. Sickling can be precipitated *in vivo* and experimentally by alterations in the ratio of combined to dissociated hemoglobin within the red cell.[17b] In the presence of high oxygen tension most of the hemoglobin exists in the combined form as oxyhemoglobin, and the red cells tend to remain entirely normal in shape and behavior. As the oxygen tension is lowered and the contained hemoglobin becomes correspondingly dissociated to the reduced form, the red cells begin one by one to sickle until finally (experimentally) 100% will assume some abnormal form. It would appear that each cell has a specific threshold for sickling determined by the percentage of reduced hemoglobin. When this threshold is reached sickling is abrupt and almost instantaneous.

Crossing the threshold in the opposite direction reverses the process. Sickled red cells when placed in contact with oxygen reassume immediately their original disk shapes and become indistinguishable from normal red cells.

Ham and Castle[11] have observed that red cells when stored *in vitro* increase progressively in volume, spheroidicity and fragility, and ultimately hemolyze. These progressive changes are believed to be caused by metabolic processes which increase the osmotically active constituents of the red cells. The authors suggest that stasis in the spleen and other organs may explain the hemolytic destruction of red cells which takes place in the active phase of this disease. The time required for samples of blood from patients with sickle cell anemia to run through an Ostwald viscosimeter was found to increase markedly when the oxygen tension of the blood was lowered to 40 mm. Hg or less. In contrast, serum or plasma from these patients, normal blood and blood from patients with other forms of hemolytic anemia exhibited no differences in the rate of flow in the viscosimeter, whether fully oxygenated or fully reduced. The suggestion made is that when any disturbance, such as an infection, leads to increased plasma viscosity or tendency to rouleau formation the resultant delay in the passage of red cells through capillaries decreases the oxygen content and initiates a vicious cycle of erythrostasis and of hemolysis.

A reëxamination of the behavior of the red cells in sickle cell disease has been made by Murphy and Shapiro[17a] (1944). From the difference observed in the rate and extent of sickling between whole fresh blood and mediums such as serum and saline solution which contain no fibrin

meshwork, they revive the old hypothesis that sickling is in some way related to the development of the fibrin net. They confirm the accepted principle that the dissociation of oxyhemoglobin bears a direct relationship to the phenomenon of sickling, with the cautious reminder that sickling and the dissociation of oxyhemoglobin may be simply parallel incidents, causally unrelated. Indirect evidence, such as the resistance to sickling of immature red cells and the invariable reversible association between sickling and oxygen asphyxia of the cells, argues for a causal relationship between the two occurrences. The sickling threshold for red cells is somewhat variable, depending on age of the cells. The evidence indicates that as the cells age the threshold becomes lower. Some cells sickle when a small part of their hemoglobin is uncombined, whereas sickling in others may require dissociation of almost all of the oxyhemoglobin. No relationships were found to variations in electrolyte ionic balance, though the level of available potassium in the cell may be of some significance in the sickling phenomenon.

Prolonged administration of 80 to 100% oxygen to patients with sickle cell anemia was undertaken by Reinhard, Moore, Pubach and Wade,[21] to determine whether the oxygen tension of arterial blood could be raised sufficiently to decrease the extent of the intravascular sickling. It was hoped that this might reduce the rate of hemolysis, with consequent lessening of the degree of anemia and with relief of pain during sickle cell crises.

Pure oxygen was given without intermission for 8 to 20 days to patients having abdominal or muscular pain, with no relief being obtained. Within a short time after the flow of oxygen was started, nevertheless, the percentage of sickled cells in both venous and arterial blood decreased decisively in the venous circulation, often from over 40% to less than 20%. This change persisted as long as pure oxygen was breathed. The oxygen content of the arterial blood became increased so that it equalled or exceeded the oxygen capacity. No consistent change occurred in urobilin excretion in urine or feces to indicate that the rate of hemolysis had been materially altered.

The most dramatic results of prolonged giving of oxygen were: (1) a fall in reticulocytes which usually began on the 4th to 6th day and which lowered the level from the initial 20 to 30% to as low as 1%, and (2) a fall in the red cell count, which usually began on the 6th to 8th days and was as great as 0.5 to 1.5 million red cells. The giving of nearly 100% oxygen thus depressed erythropoiesis—the physiologic antithesis of the erythroid stimulation produced by low oxygen tensions. After the oxygen was discontinued the reticulocytes rose again, to reach a peak of occasionally more than 50 to 80% on the 5th to 8th day, and the red cell levels began to increase coincidentally.

A critique of the methods commonly advocated for detection of the sickle cell trait has been presented by Diggs and Pettit.[8] Of the methods tested, namely the moist preparation, the gas chamber method, the moist stasis preparation, the test tube method, and moist preparations plus dyes and other chemicals, the procedure which proved most reliable and practical was the moist stasis method. In this procedure a rubber band is placed around the proximal portion of the subject's finger and allowed to remain for 5 minutes before the finger-tip is punctured to obtain a drop of blood. The blood is sealed under a cover-slip, and left up to 24 hours before the final reading is made. It is always advisable to make several moist stasis preparations from each patient to guard against artefacts, and the preparations should be made rapidly to prevent insofar as possible the exposure of the drop of blood to the air. Nevda and Rosen[18] claim that sickling in artificial circumstances can be accelerated by adding a droplet of a broth culture of normal feces to the blood placed on the testing slide.

Active vs. Latent. The active variety (known customarily as "sickle cell anemia" or "disease") is a chronic hemolytic disturbance associated with striking blood changes and obvious signs and symptoms. The latent variety, known as "sicklemia" or the "sickle cell trait," is viewed as non-productive of symptoms and free from blood changes, apart from the tendency of a proportion of the red cells in wet preparations to assume the characteristic bizarre shapes.

It has been known for some time[4a] that the red cells from individuals with sickle cell anemia change shape more rapidly and in greater numbers than those from individuals with the sickle cell trait. Sherman[22] discovered that a much lower atmospheric pressure, *i. e.*, a much lower oxygen tension, is required to produce sickling in the sickle cell trait than in sickle cell anemia. In wet preparations at pressures of approximately 50 mm. of mercury the sickling was regularly produced in the red cells from 4 patients with anemia but not at all in 3 patients with the sickle cell trait.

Sherman carried this observation to the *in vivo* situation. He filled the dead space in a Luer syringe with white oil, attached a needle and withdrew blood from patients in the usual manner. Without exposure to air about 1 cc. of the blood so obtained was injected into 2 or 3 cc. of saline formalin under oil, and examined microscopically after a few minutes allowed for fixation. Sixteen such tests on patients with sickle cell anemia showed in every instance between 30 and 60% of the red cells to be in characteristically sickled forms in the venous circulation. Eight similar tests on 6 patients with the sickle cell trait showed no sickle cells in the venous circulation in 4 instances, and only a rare sickle cell (less than 1%) in each of the remaining tests. Application of a tourniquet for 10 minutes before the test failed to show any marked effect in either class of patients. This method is presented as a quick and simple diagnostic test between sickle cell anemia and the sickle cell trait. Interestingly, arterial puncture performed in this manner in sickle cell anemia revealed up to 20% of the red cells "incompletely sickled," whereas venous blood obtained at the same time from the same patients showed between 40 and 60% of the cells to be "more definitely sickled."

Sherman found also that in sealed standing preparations an increased leukocyte concentration, a temperature elevation, and bacterial contamination each accelerate sickling, whereas saline dilution and increased oxyhemoglobin concentration retard the phenomenon. "This furnishes an explanation of the inconsistency of results obtainable with this method."

Sedimentation Rate. It is well known that there exists a definite association between the tendency of red cells to form rouleaux and their sedimentation rate. The rapidity of the sedimentation rate usually is inversely proportional to the rapidity of sickling. Bunting[2] noted that sickled red cells from patients with either sickle cell anemia or the sickle cell trait failed to form rouleaux or to sediment appreciably within 1 hour. Non-sickled cells from these same patients formed rouleaux and sedimented normally.

In 6 of 10 patients with sickle cell anemia studied by Winsor and Burch[30b] the sedimentation rates were unusually slow. Experiments indicated that this delay was linked to the degree of carbon dioxide saturation of the blood. The retardation in rate of sickling was greater as the carbon dioxide content became raised, as after re-breathing, or on exposure of the red cells to carbon dioxide *in vitro*. Conversely, inhalation of pure oxygen by the patients, or exposure of the red cells to 100% oxygen *in vitro*, accelerated the rate. The fact that the sedimentation rates of the patients became accelerated by breathing 100% oxygen meant, to Winsor and Burch, that the blood of these patients was not fully saturated with oxygen when breathing air. This led to the recommendation that when operation is necessary for these patients

an anesthetic gas which does not produce anoxemia is to be preferred.

Winsor and Burch[30b] ingeniously devised some simple tests, called the "diagnostic parameter," based upon the faculty of the sedimentation rate in sickle cell anemia to be increased by oxygen and decreased by carbon dioxide. These tests were found to be 98% reliable in the identification of sickle cell anemia. In the "aëration-tourniquet" test a tourniquet (blood pressure cuff) is applied to an arm for 6 minutes to produce venous stasis. Five cc. of blood are drawn from a vein and placed in a small vial containing an anticoagulant, stoppered, and rotated gently to avoid mixing the blood with air. A sample of the blood is set up for a sedimentation measurement immediately. The remainder of the blood is rotated in air in a small beaker or Erlenmeyer flask, to saturate it thoroughly. The sedimentation rate of this aërated blood is also determined. Within 15 to 60 minutes the difference between the rate of fall of the aërated and non-aërated samples should be greater than 20 mm. per hour if the patient has sickle cell anemia. As soon as this parameter is reached, the test need not be continued further. A similar but more difficult approach is to withdraw blood from the arm, expose half to oxygen and half to carbon dioxide in separate containers, and note the difference in rate of sedimentation.

Pathogenesis of Lesions. Changes in local organs can be attributed to the abnormal red cells, whose contours and rigidity interfere with smooth passage through narrow capillaries. As Murphy and Shapiro[17a] express it, the red cell may be flexible and elastic in its disk form' but the moment it sickles it becomes "fixed and rigid as a crystal of ice as it moves about and abuts against cells and fixed objects." The end-to-end length of the sickled form may range from 2 to 5 or more times the transverse dimension of the intact red cell, with long processes tapering from the ends. This distortion of shape and inflexibility give rise to a tendency for the abnormally shaped cells to interlock and jam within the microscopic circulation. The capillaries, sinusoids and small blood-vessels dilate, and the flow becomes sluggish. The local anoxemia which follows leads to still further deformities in cellular shapes, and soon the fine arterioles become occluded and begin to thrombose. The end of the process is local ischemia and necrosis, particularly in areas with poor collateral circulation.

Thrombosis of blood-vessels appears to be the most constant pathologic finding in sickle cell disease. In the pathogenesis of infarcts the first step is not anemia but thrombosis. Though conditioned by local anatomy, the mechanism is much the same in all organs. Death is usually due to some complication related to circulatory stasis.

Red cells are more likely to be sickled in the venous than in the arterial system. In active cases sickled cells are also found in large numbers in the liver at autopsy[1,7] and in the spleen at autopsy and at splenectomy.[25] In both of these organs the flow of blood can be slow, giving the red cells ample opportunity to discharge their oxygen.[32] Murphy and Shapiro[17b] believe that the fibrosis and hemosiderosis characteristic of the spleen in sickle cell disease may be secondary to the sickling and stasis which obtains in that organ. "The sickled cells and the stasis lead to thrombosis of vessels, with infarction and subsequent scarring and contraction."

Practically every organ of the body may be affected by the process, giving rise to a rich diversity of symptoms and signs.[30] The cardiovascular system is affected in about 90% of patients, according to Winsor and Burch,[30] who cite how a diagnosis of rheumatic or congenital heart disease was made erroneously in more than half the patients at one hospital. Lesions of the nervous system have produced paralytic changes thought to be poliomyelitis; pulmonary infarcts resemble bacterial or a typical pneumonia; involvement of the abdominal viscera,

spleen, liver or gastro-intestinal tract can mimic ruptured peptic ulcer, ruptured gall bladder or acute appendicitis, leading to surgical intervention and carrying a high mortality.

Stasney's[24] study of 12 spleens from patients with sickle cell disturbance revealed active erythrophagocytosis by the Küpffer cells of the liver, and occasionally also by the reticulum cells of the spleen. The changes in the spleen were chiefly those of abnormal distention of the perifollicular sinusoids, hemorrhage and hemosiderosis. There was a propensity for the spleen to be large in childhood, small and atrophic in adult life. A significant comment was that no salient distinctions could be found between the clinically active and clinically latent forms of sickle cell disease. The "inactive" cases exhibited the same sort of changes as the "active" ones, the differences being only in degree.

Autopsy sections from more than 150 spleens of patients with sickle cell disturbances were examined by Tomlinson.[27] No congenital abnormalities of the arterial capillary endings in the red pulp cords of the venous sinusoids, or of the pulp cords themselves were found. The histologic picture was that of chronic active hyperemia of the pulp cords, especially in the marginal zones. These were packed with lamellated sickled red cells which were believed, because of their shapes, to escape only with difficulty through the stomata of Mollier. The arterial capillary pressure accentuates the packing of the cells. Tomlinson proposed that the "pooling" and intense packing of the sickled forms in time leads to thrombosis, hemorrhage, and scarring with calcium and iron deposits, resulting eventually in a small fibrotic spleen.

The hemolytic manifestations of the disease can be interpreted as being due for the most part to the lysis and resolution of the multiple thrombi. To explain the precipitation of hemolytic crises, Murphy and Shapiro[17a] postulate that sickled cells accumulate in the circulation between crises, due to an increasing tendency of the red cells to sickle as they become older. "The concentration of the sickled form eventually becomes so high that, possibly owing to alterations in the coagulating mechanism of the blood, they suddenly precipitate out in a more or less simultaneous capillary blockage." Far greater proportions of sickled red blood cells are destroyed in a crisis than are those still normal in shape. Thus, afterwards, relatively few red cells in the sickled form remain in the circulation. During the intervals between crises hemolytic activity still goes on, the long brittle erythrocytes being broken up by the trauma of capillary passage; a small degree of capillary thrombosis may also take place continually, contributing to the sustained low-grade hemolysis. The trigger mechanism which sets off the massive precipitation of sickled erythrocyte and blockade of capillaries is presumed to be an altered state of blood coagulability. "With each thrombosis there are liberated into the circulation certain coagulating bodies (thrombin, thromboplastin) which further enhance thrombosis and accelerate the precipitation out of the circulation of sickled red cells. A self-perpetuating sickle cell crisis is thereby inaugurated which gains momentum until the major part of the sickled cells are destroyed and the blood is again rejuvenated by release into the circulation of immature normally shaped cells. The sudden liberation of coagulating bodies into the circulation accounts for thrombosis of larger blood-vessels also."

Hyperbilirubinemia as the result of the increased blood destruction can give rise to stones in the gall bladder, by much the same mechanism as leads to these concretions in other varieties of hemolytic anemia.[13] An excess of bilirubin in the bile favors aseptic precipitation of pigment in the biliary tract. Once the nucleus of a gall stone is formed, secondary factors such as stasis and infection determine its ultimate chemical composition. In most reports the stones have been described as multiple, small, greenish black and soft,

of the pigment type. Weems[29] described such stones in 4 patients with active sickle cell disease. Their ages ranged from 13 to 38 years. In 12 of 44 necropsy reports reviewed, gall stones were either found at autopsy or had been removed surgically during the course of the disease. All of these patients were in the 2nd decade or older. Cholelithiasis in a Negro should always incite investigation for sickle cell anemia.

Differentiation between rheumatic myocarditis and the cardiopathy in sickle cell anemia is not always easy in childhood. Halpern and Faber[10] have discussed this problem recently. The differentiation depends on the failure of pain in the extremities to respond to salicylates, the finding of jaundice, lymphadenopathy, or an abnormal spleen, the demonstration of anemia, reticulocytosis and sickling of the red cells, a slow sedimentation rate, diffuse cardiac enlargement, a prolonged P–R interval, and the characteristic bone changes associated with sickle cell anemia. Rheumatic carditis appears to be extremely rare in patients with sickle cell anemia.

Habitus. The physical characteristics of patients with sickle cell anemia is "often striking and at times characteristic," according to Winsor and Burch,[30] who studied 15 patients, of whom 7 were children. The changes were most marked among adults in whom sickle cell anemia had been active for many years and least marked among children and those whose disease had been manifest for only a short time. Both height and weight were less than normal. The trunk appeared short and the legs long. Occasionally the head was elongated (dolichocephaly) or tall (scaphocephaly). When the disease was severe the neck was short and the head heavy. The normal spinal curvatures were accentuated, producing marked kyphosis and lordosis. Shoulders and hips were narrow. The arms and hands were long and slender and frequently the fingers almost touched the knees as the patient stood erect. The feet were elongated to a lesser degree. In general the patients appeared frail and lacked muscular development and subcutaneous tissue. In children the chest was sometimes deep, and the abdomen often protruded sharply and markedly at the xiphoid process. The arms and legs were thin and stood out in sharp contrast to the abdominal enlargement. No abnormal habitus was observed in patients with sicklemia. The majority of adult patients showed some gonadal hypofunction. This "drepanocytic habitus" was believed to be independent of direct genetic factors and due exclusively to the continuing influence upon body growth of the anemia, influenced also by the endocrine and circulatory changes.

Race. Cardoza[3] studied the distribution of the blood Types A, B, AB and C, and also M, N and MN, in patients with the sickle cell trait, and found no marked tendency for the individuals who exhibited sickling to be concentrated in any one blood group.

Ogden[19] recently reviewed the reported cases of sickle cell anemia in the white race and added 9 examples of the sickling trait (2 having active sickle cell anemia) in 2 white families. Both mothers had the sickle cell trait; each had transferred the disease in active form to at least 1 child and the inactive form to all other children. In 1 of these families, of German stock, Negro ancestry was established. The other family was of Spanish nationality.

Examination of the racial history in all reported cases led Ogden to the conclusion that in not a single case was the descent traced far enough back to exclude beyond question a possible Negro ancestor. With the exception of 1 American family, whose ancestry was studied for but a few generations, all white persons with sickle cell anemia reported on up to the present time have been of Mediterranean origin (Greek, Italian, Sicilian and Spanish). The invasion of the Mediterranean area by Hannibal and later by the Moors, the slave trade, and the participation of Negro soldiers in European wars have all served to bring the Negro in contact with the white in the

Mediterranean area. Ogden concludes, dogmatically, from his examination of the evidence, that "presence of the sickling trait in a white person is a definite proof of admixture of Negro blood in the immediate or remote ancestry."

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GYNECOLOGY AND OBSTETRICS

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THE VAGINA

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ARTIFICIAL VAGINA. There can be no doubt that a young woman without a vagina can be an object of pity, especially if her other sexual organs are developed and she has a desire to be married. For many years gynecologists have devised operations to construct an artificial vagina. Many of these operations utilized a part of either the large or small bowel, necessarily involving some form of intestinal resection. The mortality of such operations was high enough to discourage their use although in some instances the need for a vagina was considered so urgent as to justify a risk to life. In recent years the trend has been away from operations of this type because fairly satisfactory results have been obtained by safer methods. Wharton[16] has been interested in this subject since he first performed his simple operation 20 years ago. He states that, in general, 2 methods of construction of the vagina seem to have replaced the older more complicated procedures: (1) the technique first performed by him in 1926 and published in 1938; and (2) the simpler procedure described by Frank in 1938. The former procedure is based on the principle first used by the author, that the adult vaginal epithelium will proliferate and cover a dissected space, just as the embryonic vaginal epithelium proliferates and forms the vagina in the fetus. Using this principle, one merely dissects out the requisite space for the vagina and keeps it open by a suitable vaginal form; in time the epithelium from the vestibule or introitus will spread upward and over the dissected space with normal vaginal epithelium. Frank's operation is based on the principle that, in some young women, a space can be made between the urethra and rectum by firm, daily pressure of a hard tube, without any surgical dissection or anesthetic. Satisfactory vaginas have been made in this simple manner. This is the oldest of all methods of constructing the vagina, for a description of it was published more than 100 years ago; and, probably 25 years ago, the late Dr. Howard A. Kelly remarked that he had seen a case in which the vagina had been made solely by the perseverance of the husband. In general, it is best to construct vaginas only in women who are going to use them. If the constructed vagina is not used, it will probably contract just as the natural vagina does. The best results have been obtained in young women who have been recently married or are about to be. The ultimate result is liable to be questionable in older women who have never developed normal sex life, for, with advancing years, the sexual activities of both the husband and wife are apt to be on the wane. The question arises whether one should construct the vagina in women who have no ovaries, or who have been deprived of ovarian function, or in male pseudoherma-

phrodites who have been brought up as females. The answer to these questions is found in the study of each individual patient. In young women who have no ovaries at all, a satisfactory vagina can be made and maintained if it is used often. Even in male pseudohermaphrodites Frank has successfully made vaginas which have not contracted. The chance of making and preserving a functioning vagina is distinctly less if ovarian function is absent or if the patient does not have normal sex life. In deciding whether a vaginal construction is likely to be successful in any given case, the psychic and sexual attitudes of the patient are to be considered. Many women with normal vaginas have unsatisfactory coitus and complain of dyspareunia because of nervous tension, hypersensitiveness, and sexual frigidity. If the patient with gynatresia also has these psychic and mental traits, the chance of success is distinctly decreased. The technique which he has used is as follows: A transverse incision is made across the vestibule, where the opening of the vagina should be. If there is a rudimentary vagina, the incision is made across its vertex. A plane of cleavage is almost invariably found and can be followed without difficulty. As a rule, the vaginal space can be separated in 5 or 10 minutes, largely by blunt dissection. Having prepared the desired space, the proper vaginal form is then placed in it. He has used balsa wood forms in most of his recent cases because it is light, easily cut to any shape, and so cheap that one can keep a dozen or more forms ready at all times. They can be given to the patient. Each form is covered with a rubber condom, and sterilized by soaking in bichloride of mercury solution and then alcohol. A set of these forms is ready for use when one starts the operation and the appropriate size can be used in each case. Thiersch grafts are sewed over the form by interrupted sutures of the finest catgut (0000). The form is then inserted into the dissected space, and the operation is over. The form is not disturbed for 3 weeks; by that time the grafts should have taken strongly, and most of the vaginal walls should be firmly covered by healthy skin. The use of grafts hastens the covering of the vaginal walls and decreases the formation of scar tissue, which may develop with slower epithelization. If the grafts do not take, they will slough out, and there is an excellent chance that the vagina may then be covered by the spreading epithelium from the vaginal orifice, if the forms are kept in long enough. An indwelling urethral catheter may be used or not, as one chooses. The vaginal orifice must be large enough to avoid compressing the urethra between the pubis and the form, otherwise the terminal external urethra may slough.

Regardless of the choice of material or technique of operation, unless one dissects out a large vaginal space, the result will probably be disappointing. One should see to it that the space is deep (11 to 13 cm.), wide, and that the vaginal orifice is big, admitting 3 fingers. During the months after operation, the walls tend to contract, and hence one must create a space larger than is really needed. After the vagina has been made and is covered by epithelium, it may get bigger and deeper with coitus; but this does not obviate the necessity of having an adequate space to begin with. Bleeding is not commonly encountered in dissecting out the vaginal space. If it is, it should be controlled completely, so that the grafts may lie on a dry surface. The points which are most liable to bleed are the lateral walls at the level of the broad ligaments. During the dissection, one can feel these structures like bands of thin ribbon. If they do not yield to blunt dissection, they must be cut. Otherwise they will constrict the vagina at that point and give the vagina an hour-glass shape. Also, since these areas are vascular and convey the vaginal branches of the uterine vessels, they will bleed unless they are properly handled. Such vascular areas should be ligated by transfixion. Serious hemorrhage has occurred after

operation from inadequate hemostasis in this region. During the dissection of the vaginal space, there is not much danger of injuring the rectum or bladder if the plane of cleavage is normal and if the patient has not had a similar dissection before. Between the bladder and rectum there is normally a well-defined plane of cleavage, which permits the operator to separate these organs largely by blunt dissection. This dissection can be usually carried up to the peritoneum of the cul-de-sac without any trouble at all. But if the patient has had a former dissection in the region, a previous unsuccessful attempt to construct a vagina, then this plane of cleavage is replaced by scar tissue, and the rectum and bladder are glued to each other by dense fibrous tissue. Every moment of dissection, in such a case is fraught with the danger of perforating the rectum or bladder; such dissections are always difficult, tedious and long. Injuries to the bladder and rectum are common under these circumstances. Rectal perforations are more annoying because they always introduce infection. In 1 instance, the contractions of the abdominal and perineal muscles forced the end of the vaginal form through the apex of the vagina into the rectum 5 weeks after the operation. This made a hole 4 or 5 cm. wide in the apex of the newly formed vagina and transformed what appeared to be a perfect result into a possible disaster. He closed the fistula loosely by 4 or 5 interrupted sutures of 0 chromic catgut, packed the vagina loosely with xeroform gauze, and kept a large tube in the rectum for 2 weeks. The fistula closed completely, and 1 year later the patient had a large vagina 7 cm. deep.

The experience in this type of work which has been reported by Brady[3] is unusual in that 4 different methods, determined by pertinent circumstances, were utilized to construct vaginas in 4 women in whom that organ was congenitally absent. Other surgeons have described their results in these cases, but in most instances each surgeon has recommended only one form of treatment which he has used on all the women under his care. His experience would indicate that several different methods can, if properly used, be equally effective.

The choice of methods should be based not only on the anatomic findings present, but also on the temperament, the marital status, the intelligence and coöperation of the woman one is treating. Before attempting to make a vagina a surgeon must be familiar with the normal dimensions of that organ. Most authorities stress the fact that the anterior wall is shorter than the posterior because the vagina is united to the uterus at an acute angle. The length of the anterior wall varies from 6 to 8 cm. while that of the posterior from 7 to 10 cm. What is perhaps more important than the measurements of the normal vagina is what length and circumference are necessary for the completion of the sex act satisfactorily both for the woman and her husband. This will, of course, vary to some extent with the husband, but it is certain that in many instances a vagina considerably shorter than the normal length permits satisfactory marital relations.

In Brady's first case, because of the repeated attacks of appendicitis, a celiotomy was performed. This gave him an opportunity to inspect the pelvic organs. The uterus was normal in contour, but only 2.5 cm. in length. The Fallopian tubes were rudimentary. The ovaries were normal, the right showing a moderately well-developed Graafian follicle. The appendix was removed, and the patient was then placed in the perineal position. A circular incision was made around the outer margin of the mucous membrane surrounding the perineal depression, following the line where the skin and mucous membrane meet. By blunt dissection, the tissue under the mucous membrane was spread apart. Then, in the same manner, the tissue laterally, anteriorly and posteriorly were all gradually stretched until finally there was a tunnel 7 cm. in length and of sufficient

diameter to admit 2 fingers. The small circular bit of mucous membrane which had originally covered the very immature vagina had been pushed further and further upward while this was being done, so that it finally rested on and covered the top of the newly made vagina. However, all the rest of this 7 cm. long tube was raw and covered with neither mucous membrane nor skin. Using a method similar to, but not exactly like the method described by Graves, and later by Davis and Cron, the following attempt was made to cover over these raw areas. The labia minora were dissected off from above downward in such a way as to leave pedicles large enough to furnish good circulation. The available mucous membrane in each flap was increased by splitting apart from below the surfaces of each labium minus, thus converting the large folds into flat surfaces. These flaps were then sewed deeply into the vagina with No. 0 chromic catgut. The raw areas left by dissecting away the labia minora were then covered by sewing together the adjacent skin surfaces with interrupted sutures of plain catgut. Care was taken, both in making these flaps and later in covering all the raw areas, not to injure in any way, or encroach too closely on, the clitoris. This patient was fortunate in having unusually long labia minora, for by using them, as just described, it was possible to cover over the entire lateral and most of the anterior and posterior walls of the newly constructed vagina. Usually all that can be done with the labia minora is to cover over the lower part of the lateral walls, as the small lips are seldom long enough to stretch beyond this, no matter how the pedicles are cut.

In the second case, an operation was refused. This refusal came not from the patient, but from the man to whom she had been engaged for many years, and later married. He would not consent to his fiancée being subjected to the risk of an operation no matter how slight the danger might be, so he decided to try Frank's method. The patient was given a pyrex glass rod $\frac{5}{16}$ inch in diameter, and told to press with this rod on the perineum over an area midway between the urethra and anus. At first, pressure was directed mostly backward, so as to keep from injuring the urethra. The direction of the pressure was changed at the end of a few weeks to upward and backward, following the same axis that the normal vagina takes. This patient was very intelligent and conscientious. She worked with the pyrex tube for at least $\frac{1}{2}$ hour, 2 to 3 times a day. Progress was slow, but steady. At the end of 6 weeks, there was an opening which would admit the tip of the finger for a depth of 2 cm. When a little bleeding would occur, as it occasionally would as a result of trauma, the patient stopped treatments for 48 hours. Six weeks later, the patient was able to introduce the glass rod 4 cm. She was then given a pyrex tube $\frac{5}{8}$ inch in diameter. Three months later, or about 6 months after treatment was started, 3 fingers could be introduced into the vagina which was now 6 cm. long. One might ask why not use this method always; but Brady thinks it has marked limitations. This patient treated herself for 6 months, and the treatments were definitely painful. Only her conscientiousness in carrying them out made success possible. She was never able to wear any of the vaginal forms recommended by others. She tried them, but was unable to sleep with the form in place, and could not carry out her duties as a nurse while wearing one in the daytime. When a patient has a small vagina to start with—perhaps measuring 3 to 4 cm. in length—this method would seem to be the best. Then, too, as in this case, it is all that can be tried when an operation is refused. However, when there is no vagina, or only a dimple 1 cm. in depth, few women would continue these treatments long enough to make the method succeed, and, therefore, in many instances, an operation is preferable.

In the third case Brady tried the method of Wharton which we have previously described. He made his dissection up-

ward slowly until only about 1 cm. separated the top of the newly made vagina from the large mass which he had felt on rectal examination, and which up to that time he had thought to be the uterus. This dissection upward was carried out until a vagina 7 cm. in length had been made. At this point he thought it wiser to make a lower abdominal incision and actually see the condition in the pelvis before burrowing further upward from below. After making this incision, he was surprised to see that what he had thought was the uterus was the patient's only kidney. There was no renal tissue in either renal fossa. The pelvic kidney was one-half again the size of a normal kidney. In this case there were embryologic defects in both the generative and urologic system. It has been pointed out by several writers that when there is a defect in 1 of these 2 systems, one should be on guard for abnormalities in the other. In spite of this he mistook, at least temporarily, the patient's only kidney for an enlarged uterus. If he had not recognized while operating, that he was dealing with something very unusual and had continued his dissection upward from the vagina, there is considerable likelihood that one of the large renal vessels might have been torn, with very serious consequences. In the future, in every case of absence of the vagina he will take a routine intravenous pyelogram before operating.

In the fourth case an inverted U-shaped incision was made through the skin, beginning just below the urethra and extending backward, and slightly laterally, until the dorsal end of the incision terminated 2 cm. ventrally and laterally to the anus. This incision was then continued through the subcutaneous fat for about 1 cm. Then, beginning at the ventral border, the tissue was undercut from above downward, forming a flap whose pedicle was the tissue in front, and just lateral to the anus. After turning the flap backward, the fat and subcutaneous tissue under it were carefully separated and stretched by blunt dissection until there was a cavity into which 2 fingers could be introduced for a distance of 8 cm. In carrying out this dissection, the operator took special care not to come too close to the urethra, bladder or rectum. A retention catheter had been introduced into the bladder, and a rectal tube into the anus before the operation was started and, by means of these, it was possible to tell just how closely the dissection approached the urinary and intestinal tracts. In this case, there was never any danger of the urethra or bladder being injured. However, as the dissection was carried upward, the rectum seemed to be getting nearer and nearer until only a small amount of tissue separated it from the cavity that was being formed. Because of this, the dissection upward was stopped when the cavity measured 8 cm. The steps remaining in the operation were those which would cover this cavity with epithelium and which would prevent its postoperative obliteration through contraction. The first step in accomplishing this was to carry the U-shaped flap of skin and subcutaneous fat which had been made by the original incision deep down into the cavity and sew it to the underlying tissue. No. 0 chromic catgut sutures were used for this fixation. The flap measured about 6 cm. in length. By extending the ends of the original incision practically to the anal margin, this flap might have been made 1 or 2 cm. longer. To cover the anterior and lateral walls of the vagina, the labia minora were dissected off from above downward in such a way as to leave pedicles sufficiently large to furnish good circulation. The available mucous membrane in each flap was increased by splitting apart from below the surfaces of each labium minus, thus converting the large folds into flat surfaces. These flaps were then sewed deep down into the vagina with zero chromic catgut. Care was taken, both in making these flaps and later in covering all the raw areas, not to injure in any way, or encroach too closely on the clitoris. The only raw area that remained was in the uppermost part

of the posterior wall. To cover this with epithelium a mid-thickness split-graft, measuring 11 by 5 cm., was cut from the right thigh. This graft was sewed over a Wharton "vaginaform" made of balsa wood, then introduced into the vagina in such a way that it was in close contact with the remaining uncovered area. A retention catheter was left in the bladder. The patient left the operating room in good condition. There had been very little loss of blood.

Somewhat similar to the technique which Brady used in his first case and yet differing in details is the method reported by Falls.[8] The operation is extremely simple, devoid of dangers and gives satisfactory results in the cases in which it has been used. As a rule, in these patients, the external genitalia are fairly well formed, and in many there is a slight pouch or at least a dimple in the perineum to indicate the normal location of the vagina. A circular incision is made around the edges of this dimple, creating a disk about 2 to 3 cm. in diameter. The edges of this disk are undercut, leaving the center attached to the underlying tissue. The loose connective tissue between the bladder and rectum is separated by blunt dissection, and the disk of skin is pushed inward to form the vault of the new vagina. Skin flaps about 1 cm. wide are cut from the edges of the vulvar opening by making lateral cuts at 3 and 9 o'clock, the width of the flap, and extending the incision upward and downward for about 2 to 3 cm., making 2 ribbons attached to the side of the vulva at one end which can be swung into the cavity, and the free edge sewed to the edges of the disk, forming the vault of the new vagina. The same is done on the opposite side so that there are 4 strips of vulvar mucosa 1 cm. wide, each attached externally to the edge of the vulva and internally sewed to the disk forming the vaginal vault by catgut sutures. The raw surface of the flap comes in contact with the walls of the cavity formed to permit sinking the disk, and the blood supply is established between the 2. The flaps also get a good blood supply through their attached pedicle. Epithelization occurs between these transplanted strips of mucosa, and in about 2 weeks a completely epithelized tube is formed. During healing, the pouch is packed with gauze daily, and a moderate amount of pressure is maintained. After healing is complete, the pouch will be found to be about 2 inches deep, and may be deepened by wearing a vaginal plug at night and making rather strong pressure on the disk or vaginal vault daily. The patient can be taught to do this herself.

A much better understanding of all these operations can be obtained by referring to the illustrations which accompany the original articles.

The most that is expected of any of these operations is the provision of an artificial vagina that will permit reasonably satisfactory sexual relations. Therefore, the case reported by Whittemore[17] in which a pregnancy followed one of the operations is certainly interesting and extremely rare. The patient was a woman of 21 operated upon for congenital absence of the vagina, which was present in association with a unicornuate uterus and a congenital absence of left kidney and ureter, left tube, ovary, round ligament, broad ligament and uterosacral ligament. There was also an abdominal left ninth rib. Within a few weeks after operation for the construction of an artificial vagina she became pregnant and later was delivered by Cesarean section of a full-term living baby.

At operation a No. 22 metal sound (F.) was first introduced into the bladder and held in the median line. With the left forefinger in the rectum, a 2 inch transverse incision was made midway between the external urinary meatus and the anus. By blunt dissection with the finger, the bladder was carefully separated from the rectum, until the cervix was finally felt. Much bleeding was encountered in this stage of the procedure from the perirectal venous plexus. The artificial cavity thus

created was enlarged laterally on both sides until the cervix with its external os was visualized and until the cavity would admit to its full length a vaginal glass speculum 10 cm. long and 3.8 cm. in diameter. The cervix was then seized with tenaculum forceps and pulled downward more into the range of vision. At this stage a fistulous tract was seen extending from the upper left side of the portio to the urinary bladder. Four sutures of No. 1 chromic catgut were placed through the upper and lower quadrants of the cervix and left long. Four plastic flaps were next constructed to serve as a lining for the new vagina as follows: (*a*) Two flaps of mucous membrane were constructed by partially removing both labia minora beginning at the clitoris and dissecting them down to a point just above the orifices of Bartholin's glands, including a broad pedicle at the base of each flap. Each of these was broadened out into a butterfly shape by incising the raw surface in its long diameter. (*b*) Then a flap was dissected from the inner aspect of each thigh consisting of the entire thickness of skin and subcutaneous fat. The 4 flaps were sutured together over an old-fashioned glass vaginal speculum as a form, thus constructing a tube with an orifice at its apex to fit about the cervix after the method devised by Graves. The 4 sutures of No. 1 chromic catgut which had been placed at the 4 quadrants of the cervix were threaded on a curved needle and brought through each of the 4 flaps near its apex. When these sutures were about to be tied, the glass form was removed and the entire tube was inverted, forming a lining for the artificially constructed vagina which was then packed with gauze overlaid with rubber dam. The thigh wounds were noted as reddened as early as the 3rd day, and by the 7th these began to gape. The left thigh flap had come out of the vagina and was partly necrotic. On the next day both thigh flaps had come out of the vagina and there was an increase in the purulent discharge. The perineal wound remained fairly clean and on the 11th day the edges were approximated with an adhesive bridge and the granulations sprayed with aristol powder. The labial flaps took well. On April 5, under cyclopropane anesthesia, a secondary closure of the thigh wounds was carried out. Granulating areas on the inner aspect of each thigh were curetted, the skin undermined above and below, and the skin edges freshened. Approximation of the edges was accomplished with pulley sutures and by plain interrupted sutures of heavy silk. From his experience with this operation, Whittemore would in the future use only the labia minora to line the artificial vagina and not try to employ skin flaps from the thighs. During the following 6 months the patient came to the office at bi-weekly intervals for dilatation of the artificial vagina. Coitus with normal orgasm was reported to have occurred 5 days after discharge from the hospital. Menstruation began on May 25 and lasted 4 days with a good normal flow from the vagina. A definite menstrual period occurred on June 27, and a very slight flow on August 7 which lasted only 2 days. Morning vomiting occurred several times, and on September 19 the uterus was found to be definitely enlarged and lying to the right of the mid-line. From this point pregnancy progressed normally with occasional symptoms of slight incontinence. On March 10, rectal examination showed the cervix to be $1\frac{1}{2}$ fingers dilated, but the patient was advised to enter the hospital for Cesarean operation. She was admitted on March 18 just 1 year after the operation. The indications for an elective Cesarean operation were the rather rigid, narrow artificial vagina, with congenital absence of the perineal body. There was also the question of the strength of the uterine wall because of the supposed absence of left adnexa and ligaments. Cesarean section was carried out on March 19.

VAGINITIS. Of the various types of vaginitis which are encountered, the one which has received the most attention

has been that due to the gonococcus. While the gonococcus does not infect adult vaginal epithelium, but causes a vaginal discharge by infection of the cervix, for the purpose of this review the term vaginitis will be used to indicate an inflammation of the lower genital tract, associated with a purulent discharge containing gonococci. A few years ago, many pages would have been devoted to the relative values of the various sulfonamides in the treatment of this condition. The availability of penicillin has greatly modified present-day concepts. According to Mulholland,[11] as we survey the situation regarding the use of chemotherapy and antibiotic therapy, the picture changes from month to month. Even though we see this passing parade of facts and figures, they may not impress us markedly if we do not take time to really catalog them. For instance the treatment of gonorrhea has changed greatly in the past 2 years. In the period 1940 to 1945 the percentage of cures was nearly 100 and hope was expressed that gonorrhea would be completely eradicated by means of the sulfonamide drugs. As time goes by, it is noted the percentage of cases not responding to sulfonamides is progressively increasing.

In the study of large groups of cases of gonorrhea in the Army during the past war, there is no doubt sulfonamide resistance is becoming more and more common. *In vitro* experiments show that some strains are normally more resistant than others, some being completely unaffected by the drug. Some strains appear to be naturally resistant while others acquire resistance, perhaps as a result of sublethal doses. If we assume gonococci are either sulfonamide resistant or sulfonamide sensitive, it would follow that the more people treated with sulfonamides, the more likely the most sensitive strains will be killed off and the relatively resistant strains survive. As time goes on this presents a rather poor prospect for adequate control of the disease with this drug. It would follow that all the prevalent strains in a decade or so would be sulfonamide resistant. Penicillin is not without some reactions and discomfort that may or may not be due to impurities. It has been reported that certain lots of commercial penicillin produce severe pain on intramuscular injection. On repeated injections, sterile abscesses containing insoluble material with no antibacterial activity have been reported. With increase in potency in units per mg., there is a corresponding decrease in the pain produced. Of the 3 sites of injection (buttocks, triceps and deltoid), the least amount of pain results from injections into the buttocks. Reactions of the allergic or serum sickness type are being reported each month. There is great difficulty in determining the source responsible for the allergic reaction. Penicillin as presently employed is not a pure product. Until pure crystalline components constituting crude penicillin can be activated in sufficient quantities to permit their use therapeutically, incrimination of penicillin allergic responses must be held in abeyance.

The data compiled by the Public Health Service on the treatment of gonorrhea by penicillin have been reported by Heller,[9] who states that the investigations were made by medical officers in charge of several hospitals established by state and Federal agencies for the intensive treatment of venereal disease. Two treatment schedules were studied: 1 that could be completed in 2 hours and another that required 3 hours. There were 396 patients studied, 248 white and 148 colored, 108 male and 288 female. In all patients the diagnosis was confirmed by a positive culture; 83% of the patients were observed 10 days or longer, 17% from 6 to 9 days. To be regarded as "cured" the patient had to be clinically and bacteriologically free of infection, *i. e.*, without signs or symptoms and with 3 or more cultures—all negative—during the observation period. The 2 hour schedule was administered to 255 patients. They received 200,000 units of sodium penicillin dissolved in 6 cc. of

water in 3 intramuscular injections; at zero hour, 50,000 units (1.5 cc.), at 1 hour 50,000 units (1.5 cc.) and at 2 hours 100,000 units (3 cc.). The 3 hour schedule administered to 141 patients called for the same total dosage administered intramuscularly as follows: at zero hour 40,000 units (1.3 cc.), at 1 hour 40,000 units (1.2 cc.), at 2 hours 40,000 units (1.2 cc.) and at 3 hours a final injection of 80,000 units (2.4 cc.). No patient showed evidence of toxic reaction, and although the injections were given at hourly intervals, the very short span of treatment was most acceptable both to the patients and to the medical and nursing personnel. Among the patients observed 10 days or longer, 94% were cured on the 2 hour schedule and 96% on the 3 hour schedule. Although there were no significant differences, the rate of cure on the 2 hour schedule was higher in the colored than in the white, higher in the female than in the male, and higher in the previously untreated than in the previously treated. In the small group of patients who apparently do not respond to treatment the diagnosis of gonorrhea may be in error, as shown in re-check of the organism on sugar fermentation mediums. Certain of the failure cases may require quite large amounts of penicillin given over a longer period of time and for this purpose may require hospitalization. But thus far research by the Public Health Service has not detected strains of gonococci which remain persistently resistant to penicillin.

According to Perkins and Brewster,[12] the application of penicillin seems to be the outstanding advance to date in the therapy of gonorrhea, the most frequent of all venereal infections. The results of treatment in both sexes, with or without complications, are so striking that all other forms of therapy now seem antiquated. There are enough failures of treatment to necessitate careful repeated follow-up examinations, which should include at least 4 or 5 cultures and smears for the gonococcus. The optimum treatment schedule has yet to be worked out, but the underlying principles seem to aim at producing penicillin blood levels high enough to accomplish the desired results over a relatively short period rather than lower blood levels for a prolonged period. The rationale of this is twofold: the goal is to develop an ambulatory treatment that can be completed at 1 clinic session; and experience seems to show that dosage schedules that achieve these high penicillin blood levels produce a larger percentage of satisfactory results. Because of the well-known repressive action of the drug on the spirochetes of syphilis, blood serologic tests should be taken 6 weeks after treatment and should be repeated in 3 months. Two hundred and thirty-four courses of treatment were given to 200 patients with either proved or suspected gonorrhea. Those treated on suspicion without any particular clinical or laboratory evidence of gonorrhea were women who had been named as sources of infection by men with gonorrhea; in most cases these were military contacts. The largest group of patients in the series received 100,000 units of penicillin. In all but 6 of these patients, who were treated according to the schedule of 20,000 units every 2 hours for 5 injections, the results were satisfactory. Forty-one patients were treated with 150,000 units of penicillin. All of these were given 50,000 units every 2 hours for 3 doses. The results were 100% satisfactory. This schedule seemed to give the best results, which is surprising in view of the fact that the 44 cases treated with 200,000 units showed failures in 4, a rate of 9%.

The report of Cohn, Taylor and Grunstein[5] is based on a series of 108 patients treated with penicillin. Of these, 101 had failed to respond to at least 2 courses of various sulfonamides; the remaining 7 patients showed a definite sensitivity to sulfonamide compounds. Of the total of 108 patients, 99 promptly became bacteriologically negative after 1 course of penicillin treatment, and 9 by the administration of a second course of penicillin.

The results obtained by the administration of various amounts of penicillin point to the fact that a minimum total dosage of 100,000 Oxford Units injected intramuscularly in divided doses, is both necessary and sufficient for bacteriologic cure. The minimum total period of time required for successful therapy was found to be 6 hours. Smaller doses of penicillin and shorter total time of treatment were adequate in individual cases, but this type of therapy cannot be recommended. After their discharge from the hospital, 81 of the total of 108 women were followed up for an average period of 43.6 days, during which an average of 3.5 examinations were performed. Fifteen patients were found gonococcus positive after an average follow-up of 51.7 days. It may be assumed for various reasons that all these patients represent reinfections rather than recurrences. No serious toxic symptoms appeared in this group of patients treated with penicillin.

An interesting study on the *vaginitis of childhood* has been made by Rice, Cohn, Steer and Adler[15] and, although the work was done before penicillin became available, it is worthy of review at this time. It was arbitrarily agreed that children to be regarded as cured must be clinically normal and that at least 7 consecutive smears and cultures made during a period of at least 16 weeks must show no gonococci.

Forty-one children were observed who were not given any form of treatment. Some recovered within a short time, 54% by the 10th week and 87% by the 28th week after the beginning of observation. This is definite evidence that spontaneous cure of the disease may occur in the majority of patients. Thirty-three children were treated with estrogenic substances (amniotin and diethylstilbestrol). The 12 (36%) who were cured were treated for an average of $54\frac{1}{2}$ days. Comparison with the control series showed that bacteriologically cure was no more frequent among patients given this form of therapy than among the untreated patients. However, definite clinical improvement followed shortly after the institution of treatment. Fifty-three children were treated with sulfanilamide. The 23 (43%) who were cured were treated for an average of $9\frac{3}{5}$ days. This drug gave definitely better results than would be expected without treatment.

As a result of the foregoing observations, it seems likely that many of the epidemics of vaginitis reported in the past were not due to the gonococcus. There is further evidence that this infection is not as contagious as has been heretofore believed. In the vaginitis ward of a hospital there were children with acute gonococcic vaginitis, children who were cured and being observed for proof of cure and others who had been admitted to the ward for observation but were later shown not to be infected. No restrictions were placed on these children, and they were not isolated one from another. Over a period of years no instance of disease in the noninfected children was observed. Furthermore, during 2 different periods of several months all the children in the ward used the same toilets without causing new infections. As many as 8 infected children with profuse vaginal discharge were placed on the same toilet in rotation, and several cultures were made of material taken from the toilet seat. On only 1 of 18 occasions were gonococci recovered, and then only a few colonies could be found in 1 culture. As far as we know, no instance of transmission of the disease by way of the toilet seat has ever been proved. Investigation of parents and siblings revealed that the probable source of infection in the majority of cases was an infected adult in the home. Of a group of parents who prior to examination believed themselves healthy, 50% were found to be infected. Another important method of transmission that was disclosed is associated with sexual curiosity and experimentation. Of infected girls between the age of 6 years and puberty, 35% admitted sexual contacts. It may be concluded that the transmission of the disease requires inti-

mate contact between infected adult or child and non-infected child. Contaminated fomites, such as rectal thermometers, enema tips, diapers, towels and linen may possibly be factors in the spread of the disease. It is recommended that children not be excluded from school because of gonococcic vaginitis except during the stage of profuse discharge. Children who must be hospitalized because of complications or the occurrence of concurrent diseases need not be placed in separate isolation wards but may be treated for the complicating factor without regard to the vaginitis. Treatment with sulfathiazole would soon make such children non-infectious. It must be remembered that during the first few days aseptic technique should be practiced by the nursing staff. In the care of infected children, instruments and fingers come in intimate contact with the genitals of the patients and if not thoroughly cleansed may possibly infect another child.

While gonococci cause an endocervicitis and little actual vaginitis in the adult, the *Trichomonas vaginalis* causes a vaginitis and little if any endocervicitis. According to Brady and Reid[4] the characteristic lesion is most often seen in the upper posterior part of the vagina just behind the cervix. One not infrequently sees in this area minute red spots, giving the upper posterior vaginal wall a strawberry-like appearance. The trichomonas is a protozoon with actively moving flagella at one extremity and an undulating membrane. The latter cannot always be seen as easily as the flagella. These organisms are larger than an ordinary pus cell and yet smaller than the epithelial cells which line the vagina and which can always be seen in vaginal smears. The trichomonads are very motile. Under the microscope their flagella can be seen moving rapidly. The technique for demonstrating these organisms is as follows: The gloved finger is inserted high in the vagina behind the cervix and a drop of the secretion taken on the gloved finger. This is mixed with a little normal salt solution and the preparation examined at once. The organisms can be seen under low or high grade power. It is not necessary to stain them. In some women, smears examined in the intermenstrual period may fail to show the organisms, while those taken immediately before or after a period will reveal them. This is what often happens. A woman comes to a doctor complaining of a leukorrheal discharge. He thinks of endocervicitis, polyps, carcinoma and many other gynecologic conditions as being the possible cause of the discharge. He at once puts green-soap or some other lubricant on his fingers and makes a pelvic examination. After his examination has failed to demonstrate any of the conditions mentioned above he then thinks of the possibility of a vaginitis due to the *Trichomonas vaginalis*, takes smears, looks at the material under the microscope and fails to see the organisms. This may be due to the fact that the green-soap on his fingers killed the trichomonads which were in the superficial tissues and by which the diagnosis could have been made. It is advisable to take smears for *Trichomonas vaginalis* as the first step in the study of all patients complaining of leukorrhea, pruritus and dyspareunia. Another factor that sometimes interferes with the diagnosis of trichomonas infections and also that of gonorrhea is that many women through a natural sense of cleanliness will take a douche immediately before coming to a doctor's office and thus prevent the physician from making the diagnosis. Although there have been many treatments recommended for *Trichomonas vaginalis* vaginitis, they may be divided into 2 groups based on what it is hoped the treatment will accomplish. In the first an effort is made to destroy all the protozoa by the use of antiseptics. In the second group of treatments less attention is paid to antiseptics than to measures which it is hoped will restore the normal defenses of the vagina. In general, this defense mechanism of the vagina is believed to depend primarily on 3 conditions: (1) that the vaginal

secretion remains at its normal low pH; (2) that the Döderlein or vaginal lactobacilli are present in sufficient numbers to form lactic acid and finally that there is sufficient carbohydrate, perhaps in the form of glycogen, in the vaginal epithelial cells or spaces between the cells to afford adequate nourishment for the continued growth and activity of the Döderlein bacilli. Being rather discouraged by the results obtained from the numerous preparations that had been recommended for the treatment of vaginitis due to the *Trichomonas vaginalis* Brady and Reid decided to try to build up the vaginal defense by carrying out several measures at once. Not only was nourishment suitable for the growth of Döderlein's bacillus to be introduced into the vagina but also viable lactobacilli. As soon as the diagnosis is made a bivalve speculum is introduced into the vagina, the cervix inspected for complicating endocervicitis and Skene's and Bartholin's glands inspected for possible involvement. The vagina is then dried with cotton and 2 lactobacillus tablets inserted high in the vagina in the posterior fornix behind the cervix. The vaginal orifice is then plugged with a tampon of non-absorbent cotton. When the patient returns on the next day the tampon is removed, material taken from the vagina for microscopic study and the treatment carried out on the preceding day repeated. On this second visit practically every patient will report that the itching has been much less, and it is very unusual to be able to demonstrate organisms at this time. Such office treatments are repeated daily for from 4 to 6 days. The patient is then told to insert 1 lactobacillus tablet high in the vagina each night. She is told to take douches only if she becomes uncomfortable from unabsorbed particles of the tablets coming out of the vagina and causing irritation. A white vinegar douche (5% acetic acid) is recommended in a strength of from 2 to 4 tablespoonfuls to 2 quarts of water. Two douches a week are usually sufficient. This home treatment is continued from 3 to 6 weeks and longer if necessary. However, if the organisms promptly disappear and show no immediate tendency to recur, the tablets need be used only every other night. It is especially important that they be used while the patient is menstruating, as that is, of course, the time when the vaginal defenses against the trichomonads are weakest. They are enthusiastic about the prompt results which they have obtained with the lactobacillus tablets, and have had numerous patients go without treatment for from 3 to 6 months in order to prove that they have really been cured of the infestation. Nevertheless they feel that every woman who has had a trichomonas infection should continue for considerable time to take either acetic acid douches or to use the lactobacillus tablets for a few days each month around the time of the menstrual period. In fact, no matter what treatment is carried out for vaginitis due to the *Trichomonas vaginalis* it should be repeated at each menstrual period for from 6 months to 1 year.

The type of vaginitis which is seen in elderly women and usually termed *senile vaginitis* is due to a lack of female sex hormone. According to Abarbanel, Aranow and Goodfriend,[1] this syndrome, which is so frequently accompanied by vulvar pruritus, is a part of the process of aging of the individual, and the entire patient must be treated, and not just one of her symptoms. Other constitutional and local causes must be excluded. Supportive treatment includes a well-balanced, properly cooked diet, rich in vitamins and minerals, as well as small doses of desiccated thyroid. At first the patient is instructed to douche twice daily while lying flat on her back in the bathtub, using 2 quarts of a lukewarm solution containing 3 to 4 ounces of ordinary household vinegar. If relief is not evident in 2 to 4 weeks, local therapy with an ointment containing 5 mg. of diethylstilbestrol per ounce is started, as this is the most effective and efficient means of administration. Approximately enough ointment to contain

0.5 to 1 mg. of diethylstilbestrol is rubbed thinly over the vulva once a day, while a small amount is smeared lightly over a vaginal cotton tampon before the latter is inserted. Acid douches are continued. When kraurosis vulvæ is pronounced, it may take several months to achieve complete relief. Pelvic examinations should be performed frequently to rule out a possible damming up of secretions, such as pyocolpos or hematometra. Uterine bleeding may occur at infrequent intervals, but it rarely is alarming. Sufficient ointment to contain 4 to 8 mg. of testosterone may be substituted once or twice a day for 1 week out of every 4. In fact, when the itching is extremely pronounced about the folds of the clitoris, testosterone ointment should be used daily, for this steroid seems to stimulate the surrounding glands to produce an oily secretion which serves to relieve the dry itching skin.

In the use of stilbestrol it should be remembered that there may be some unpleasant side reactions, the most common one being nausea. Careful study of these patients has brought to light several important factors, each of which must be carefully evaluated. The dose level is of prime importance. A few years ago it was reported from this clinic that the higher the dosage the greater was the incidence of side reactions. This has been amply confirmed. Equally important in evaluating nausea is the patient herself. Analysis of the latter reveals that 2 broad groups are recognizable. The first group is composed of patients in whom nausea may be considered as being incidental in nature. It includes those who are unable to take any type of oral medication including placebos. This group also embraces those patients who become nauseous with their flushes, for not infrequently the flushes may be accentuated at the start of treatment, thus aggravating the nausea. The nausea disappears as the flushes are relieved. These women are particularly liable to be nauseous on arising, a sort of "morning sickness" of the climacteric. The second group comprises those patients in whom the nausea may be considered as a true side reaction. In this group the incidence of nausea is clearly related to the dose level. Further study disclosed that patients with a previous history of sensitivity to fried and fatty foods are much more prone to develop nausea at a given dose level than those with a previously negative gastro-intestinal history. This was shown to be statistically significant. Just why patients with a suggestive "gallbladder syndrome" are so much more likely to develop nausea when receiving diethylstilbestrol or estradiol remains to be clarified. It has been reported that estrogen may bring about a delay in the emptying time of the gallbladder. Such an effect, if confirmed in the human being, would serve to explain the increased nausea in the "gallbladder syndrome" patients, for they would tend to be more sensitive. The management of these patients should include administration of cholagogues. Two teaspoons of magnesium sulfate once or twice a day are usually effective without causing cramping or diarrhea. Mild mercurous chloride, crude bile salts or purified bile acids may also be used. The nausea experienced by patients with a previously negative gastro-intestinal history does not usually present much difficulty. Its incidence may be considerably reduced by starting with a small dose level, 0.1 to 0.25 mg., and then raising it gradually if necessary. Administration of cholagogues is also helpful. With the majority of these patients the nausea is usually transient, disappearing in spite of continued treatment. A word should be said about dosage. One mg. of oral diethylstilbestrol daily is approximately equivalent to injecting either 1 mg. of estrone (theelin) daily or 1 mg. of estradiol benzoate about every 3rd day. Clearly, then, a daily dose of 1 mg. is a relatively large dose, especially when it is recalled that, in contrast to estradiol and estrone, stilbestrol is not inactivated by the liver. Consequently a much higher amount of active estrogenic substance is

available to the body. Furthermore, it takes from 3 to as much as 12 days for the human being to excrete a given dose of diethylstilbestrol in contrast to the 2 or 3 days required for estradiol and estrone. In short, a cumulative effect occurs, so that even 0.1 mg. daily of oral diethylstilbestrol is not such a small dose after all.

Vaginal Medication. The value of vaginal medication has often been questioned since it would seem that the length of contact of the medicament with the tissues must be brief. An interesting study of this subject has been made by Rakoff and Casper,[14] who state that, when properly administered the vaginal douche is admittedly an excellent method for bringing heat to the inflamed tissues or for cleansing the vaginal tract. It needs only to be added that as a method for medicating the vagina the douche is an inadequate one indeed, since the solution remains within the vaginal tract for so brief a time and what little medicament remains is rapidly diluted by the vaginal and cervical secretions and soon drained away. The swabbing or painting of the vagina offers some advantages over the douche in that the medication can be more thoroughly applied and often in higher concentration than is possible with the douche. Tampons saturated with medicated solutions or introduced into the vagina after the medication has been applied help to prevent the "messiness" which occurs when such solutions drain from the vaginal orifice, but since most of the medicament soon becomes absorbed in the body of the tampon, it is doubtful whether the tampon prolongs the period of useful medication. Relatively scant attention has been given to the use of water-dispersible jellies in the treatment of vaginal infections despite their widespread intravaginal use for contraceptive purposes. This is rather surprising since the jelly has much to commend it as a vehicle for intravaginal medication. Essentially, the jelly is a mixture of gums. It can be made water-dispersible so that it will mix easily with the vaginal secretions. Within the jelly most any type of medicament can be intimately incorporated in various concentrations. After unsatisfactory experimentation with various techniques of demonstrating the manner in which jellies and other types of medication behave after introduction into the vagina, the value of roentgenology with regard to this problem was anticipated and subsequently confirmed. For this purpose they employed a water-dispersible jelly made up with a tragacanth-acacia-glycerin base adjusted to pH 4.5 with acetic acid. Into this was incorporated a radiopaque substance, such as barium sulfate (15%) or lipiodol (12.5%). When 5 cc. of the jelly was introduced into the posterior fornix under fluoroscopic control, the jelly was seen to spread at once over the cervix and into the lateral fornices while a small amount spread down towards the middle third of the vagina. In this case, films made at 30 minutes and at 1½ hours show that, although the jelly was slowly spreading, most of the opaque media still surrounded the cervix despite the fact that the patient was permitted to walk about. Generally, the jelly appeared at the introitus in from 1½ to 4 hours, but as late as 12 hours a considerable proportion of the jelly was still present, spread over the mucosa. Even when the jelly is purposely introduced into 1 of the lateral fornices, it is interesting to note that it spreads to cover the cervical os—a factor of particular importance from the contraceptive standpoint. Also, when the jelly within the vagina is mechanically agitated with a large test-tube to simulate coitus, it is noted that although further spreading is facilitated the jelly is sufficiently cohesive to remain as a good cervical barrier. The amount of jelly employed is a factor of importance which must be determined by the purpose for which it is to be used, the capacity of the vagina, its elasticity, and the degree of relaxation of the surrounding tissues. Although 5 cc. of jelly appears sufficient to act as a good cervical barrier even after 30 minutes, better spreading is obtained in the parous vagina

with 10 cc. In multipara with marked relaxation of the vaginal walls, the vaginal vault can easily retain 20 cc. and as much as 45 cc. will remain in the upper two-thirds of the vagina and will be retained in the vaginal tract even when the patient assumes the erect posture.

For several years they have successfully employed various types of water-dispersible jellies in the treatment of certain vaginal disorders. Buffered acid jellies were water-dispersible with pH ranging from 2 to 5 and were employed in the treatment of various specific bacterial infections, but particularly for the so-called "non-specific" bacterial infections. In most instances the latter result from disturbances in vaginal biology associated with reduced vaginal acidity, thus favoring the replacement of Döderlein's lactobacilli by a variety of other organisms. In a majority of these cases a jelly with pH 4 proved most efficacious. Acid jellies containing stilbestrol or the natural estrogens were particularly beneficial in the treatment of vulvovaginitis in children, both gonococcal and "non-specific," and also in atrophic vaginitis in menopausal women or younger women with ovarian deficiencies. Sulfonamide jellies containing various concentrations of the sulfa compounds are well tolerated and the absorption of the drugs from the vagina is very slow. The results by this method are not as good as when the sulfonamides are insufflated into the vagina as a powder. As a form of medication the authors found suppositories in general to be inferior to jellies. In most instances the size of the suppository is small as compared with the capacity of the vaginal tract; the suppository is comparatively heavy and often gravitates to the lower part of the vagina before it has melted. The introduction of medicaments in powder form has become very popular in recent years. In the treatment of vaginal trichomoniasis especially, the "dry" method of treatment is preferred, usually consisting of the introduction of trichomonadicidal chemicals in a drying base such as kaolin. For this method of medication one may use compressed tablets, large gelatin capsules, or the powder may be blown into the vagina with an insufflator. They have found the latter to be the only really efficient method of completely covering the vagina with a powder. Although some of the powder thus introduced begins to be washed out within a few hours, much of it usually remains *in situ* for 24 hours or more.

The use of powders in the vagina should be a harmless procedure, but Martland[10] reports 2 deaths occurring after vaginal insufflation of medicinal powders for the relief of pruritus vulvæ. In the first case a virginal and cribriform hymen played an important rôle in the causation of the air embolism. The doctor, believing he was spraying powder over the external genitals, must have unknowingly held the glass tip of the insufflator directly over the largest opening in the hymen and have blown powder and air into the vagina. The air entrapped in the vagina was forced into the uterine cavity. The exact point of entry of air into the venous circulation could not be demonstrated. The fact that the patient had just passed a menstrual period favored engorgement and distention of the uterine veins. The second woman was 6 months pregnant. In this case the metal end of the insufflator must have rested against the soft cervix of the pregnant uterus and considerable powder and large amounts of air were blown directly into the uterine cavity. The main purpose of the insufflators is to dilate and smooth out the folds in the vagina so that the powder may be sprayed on the mucosa. It is obvious that the sudden blowing up of the vagina with air under excessive pressure is not without danger. The engorgement of the endometrium around the menstrual periods, open veins after uterine curettage and the soft cervix of a pregnant uterus all might favor air embolism during such insufflations.

Posterior Vaginal Hernia. The changes in the normal anatomy of the pelvic floor which permit a herniation of the cul-de-sac into the upper vagina pro-

duce a condition which has been described in the literature under the titles of posterior vaginal hernia, enterocele, cul-de-sac hernia, Douglas' pouch hernia, enterocele and high rectocele. It is characterized by the protrusion of a peritoneal sac through the cul-de-sac of Douglas which dissects its way between the vagina and the rectum and presents as a mass in the vagina. In discussing the mechanism of this defect Dixon[7] states that it is of rare occurrence because the obliquity of the pelvic cavity allows the force of intra-abdominal pressure to expend itself to some extent on the bladder, symphysis and anterior abdominal wall, thus protecting the pelvic floor. The strain and trauma of childbirth are of importance in the etiology. But some congenital defect must play an important part since the condition is so rare in multiparous women, but nevertheless occurs in those who have not borne children. The symptoms complained of by the patient are a sense of weight and pressure in the pelvis, and a variable degree of bulging in the vagina. Where the sac is well developed, a mass may protrude from the vagina on standing or straining, which usually disappears when the patient lies down. Many of the reported cases had had previous operations for repair of the pelvic floor with a recurrence of the bulging as soon as the patient got up. This is always suggestive of such a hernia. Examination with one finger in the rectum and one in the vagina will differentiate the condition from a rectocele, which may coëxist, as it will show that all the protrusion is not due to the rectocele. In cases where rectocele does not exist this maneuver helps to establish the fact that the protrusion originates behind the cervix, and if the patient is asked to strain, it will determine the presence of abdominal contents in the protrusion. The treatment of this condition is surgical, since palliation by pessaries does not afford relief. The same principles apply here that apply to the treatment of hernia elsewhere, namely isolation of the sac, disposition of the sac and closure of the point of egress of the hernia from the abdominal cavity. There are 2 avenues of approach. The abdomen may be opened, the sac inverted into the abdomen and disposed of, and the cul-de-sac obliterated. Such an approach may be necessary if the abdominal contents are adherent in the sac but where this is not necessary, the vaginal approach is more desirable. This consists in opening the posterior vaginal wall from the cervix to the perineum, dissection of the sac from the rectum and surrounding tissues, ligation of the neck of the sac and excision of the excess, anchoring the stump behind the cervix and obliteration of the cul-de-sac by approximating the uterosacral ligaments. The remaining tissues are approximated and a high perineorrhaphy completes the operation.

Phaneuf[13] states that a hernia of this type may follow vaginal hysterectomy for the treatment of prolapse if the cul-de-sac is not closed. This may be prevented by approximating the uterosacral ligaments to each other in their entire length when performing vaginal hysterectomy. In addition to the 2 methods of treatment which have previously been described, he suggests the use of colpocleisis, or closure of the vagina, when the hernia is large and there are contraindications to laparotomy.

Tumors. The rarity of *vaginal myomas* is pointed out by Bennett and Ehrlich[2] who found only 9 cases among more than 50,000 specimens in a gynecologic laboratory to which they have added 3 additional cases. Most of the reported cases are in white women contrasting strikingly with the racial incidence of uterine myomas which are almost 3 times as common in colored women. The tumor usually presents in the vagina as a non-pedunculated ovoid mass covered with smooth intact mucosa and has no characteristic appearance which would differentiate it from a vaginal cyst or cystocele. In this series the tumors were usually small, varying from 1.5 to 4.5 cm. in the greatest diameter, although very large tumors

have been reported by others. The lesion is usually single and may develop at any point in the vaginal wall, although more than half of the tumors reported have been on the anterior wall. They are firm in consistence and the symptoms depend on the size and location of tumor. Pressure on the urinary organs will give urinary symptoms, either retention or frequency, while some patients complain of a protruding mass of dyspareunia. If degenerative changes have occurred in the overlying mucosa, there may be a vaginal discharge or bleeding, at times simulating a malignant tumor. The treatment of this condition is simple excision which is usually easily carried out.

Another rare tumor is *sarcoma* of the vagina which also seems more common on the anterior vaginal wall. In presenting 2 of these cases Diehl and Haught[6] state that the tumor is friable, bleeds easily on manipulation, grows rapidly, soon filling the entire vagina and presenting at the introitus as a polypoid mass of necrotic tissue. Microscopically the tumor is composed of an edematous, myxomatous stroma through which are dispersed, in varying degrees, spindle and stellate cells. Bloody vaginal discharge is usually the first symptom, frequently associated with pain and itching of the vulva and vagina. As the condition progresses, pain becomes more intense because of pressure. The pelvic mass first encroaches upon the bladder, resulting in frequency, incontinence and infection. Hematuria often develops indicating involvement of the bladder mucosa. Later the rectum becomes involved causing tenesmus, constipation and rectal bleeding. Cachexia, anemia and edema of the lower extremities are terminal signs. Distant metastases are extremely rare, the lung and kidney being the only 2 sites reported. The treatment of this condition is most discouraging. Because of its extremely malignant nature and rapid growth, the tumor is soon beyond the realm of response to surgery or irradiation.

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PHYSIOLOGY

PROCEEDINGS OF

THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA

SESSION OF APRIL 15, 1947

Preliminary Tests of Sudden Upward Acceleration on Sitting Men. D. T. WATTS, PH.D., E. S. MENDELSON and A. T. KORNFIELD (Aero-Medical Equipment Lab., Naval Air Exptl. Station). Of aviators attempting bailouts from fighter planes during the war 18 to 25% were fatally injured due to the difficulty of successful emergency escape. The most feasible method of separating the pilot from present planes utilizes an ejectable seat, driven up rails by a powder exploded catapult. Such seats were developed by the Germans and improved by the British, for use up to about 20 "G". The American Army and Navy have been collaborating in their perfection.

The test subject is fastened into a pilot's seat at the base of a 105 foot test tower, up which the seat may coast, but down which it must be lowered. Ejection guns for use in aircraft are mounted between the seat and the base of the tower. The time phenomena measured are pressure in the gun, seat displacement, muscular tension, and accelerations on the seat and on the subject's head, shoulder and hip. Performance of a number of catapults has been tested with dummies. Correlations of ballistic and dynamic phenomena have led to the development of catapults capable of producing the highest safe velocities with a given length of stroke, or given velocities with the shortest safe length of stroke.

Motion pictures of subjects using arm rests, or a face-restraining curtain pulled down by the hands, show some incipient hazards of upward ejection. The face curtain appears much safer. The dynamic response in the coupled system, man-cushion seat, points to a minimum safe time interval of 0.013 second for the application of acceleration. Acceleration levels applied to the system by the catapult in shorter periods of time result in higher levels on the man. A new seat catapult has been developed at NAES for ejection at the most rapid rate of acceleration which will not result in a dynamic response factor greater than one, and which will induce maximum accelerations only 75% of those in former systems.

Glycolysis of Different Hexoses in Brain Tissue in Connection With the Turnover of Adenosinetriphosphate (ATP). OTTO MEYERHOF, M.D. (Dept. of Physiol. Chem., School of Medicine, Univ. of Penna.).

Brain tissue as well as tumor tissue glycolyzes glucose much faster than fructose. But in cell-free enzymatic extracts both are metabolized with equal speed, which is moreover much higher than the glycolysis of tissue slices. (Q_{LA} about 50 instead of 15 to 10 in brain slices.) On the other hand the glycolysis in a homogenate of brain, which contains the disintegrated cell structures, resembles closely that of slices as well in the absolute value of Q_{LA} as in the difference of glucose and fructose.

These facts are explained by the distribution and affinities of two enzymes concerned with the turnover of ATP: hexokinase and ATP-ase. The latter enzyme is strongly adsorbed on the structural elements. In the complete homogenate it is ten times as concentrated as in the centrifuged extract, where the particles are removed. The concentration of added ATP in the homogenate drops to very low values in a few minutes because of the high activity of ATP-ase. The rate of glycolysis is mainly controlled by the

reaction between the sugar and ATP in presence of the enzyme hexokinase. First, the speed depends over a wide range on the concentration of ATP; secondly, the influence is different for glucose and fructose. With higher ATP concentrations (over 20γ labile P of ATP per cc.) the affinity of glucose and fructose is the same, but for low ATP concentrations (2γ to 8γ labile P per cc.) the affinity for fructose is much less. In this case the rate of glycolysis of fructose is much smaller than for glucose. To prove this point in tissue extracts, it is necessary to use very little hexosediphosphate for priming the reaction; otherwise the excess of hexosediphosphate serves as P donor for rephosphorylation of ATP.

From these experiments it follows for the living brain- or tumor cell, where the different behavior of glucose and fructose is quite outspoken, that the active concentration of ATP responsible for the turnover is surely quite low, and amounts to only some per cent of that found by chemical analysis. Probably the bulk of ATP is either separated from the reacting sugar by cell interfaces and immobilized in this way, or it is bound to inert protein and therefore unable to react.

Experimental Metastasis of Frog Carcinoma. BALDUIN LUCKÉ, M.D., and H. G. SCHLUMBERGER, M.D. (Dept. of Pathology, Univ. of Penna.).

Vestibular Disturbances Produced in Animals by Streptomycin. JOSEPH E. HAWKINS, JR., PH.D., and CHARLES W. MUSHETT (Merck Institute for Therapeutic Research, Rahway, N. J.).

The chronic neurotoxic action of streptomycin in rabbits, dogs and cats is similar to that occurring in some patients receiving prolonged treatment with streptomycin. In animals a gradual loss of nystagmus in response to rotation occurs during treatment with large doses of the drug by subcutaneous injection. This effect has been studied quantitatively by electrical recording of pre- and post-rotational nystagmus in unanesthetized animals. The rate and duration of both optokinetic and vestibular nystagmus are reduced, and in the rabbit both types of nystagmus may be abolished. In the cat the loss of nystagmus may progress even after the drug is discontinued. No recovery of nystagmus has occurred in animals observed for 4 months after the drug was stopped.

In addition to ataxia and other disturbances of equilibrium which occur in all 3 species, cats show difficulty in maintaining fixation upon a single object in the field of view.

Although histological studies have thus far revealed no lesions in the nervous system attributable to streptomycin, the experimental results suggest that the streptomycin may act not only on the peripheral vestibular apparatus, but also possibly upon the vestibular nerve, the medullary vestibular centers, and cerebellar and mid-brain centers. Electrophysiological studies of auditory function show that streptomycin decreases the responsiveness of both the cochlea and the auditory nerve.

The behavior of dogs and cats which had received streptomycin for 2 to 3 weeks is illustrated in a motion-picture film. This shows the changes in gait and posture occurring in dogs, the animals' inability to maintain their balance on a tilt-table, and the absence of nystagmus after rotation. Even more dramatic disturbances of equilibrium are shown in cats.

BOOK REVIEWS AND NOTICES

MODERN MANAGEMENT IN CLINICAL MEDICINE. By F. KENNETH ALBRECHT, M.D., S.A., Surgeon, U. S. Public Health Service; Kansas State Tuberculosis Consultant; formerly Clinical Director, U. S. Marine Hospital), Baltimore, Md. Foreword by ALPHONSE MCMAHON, COMMODORE, MC, USNR, Chief of Medicine, U. S. Naval Hospital, Bethesda, Md. Pp. 1238; 237 ills.; 11 color plates. Baltimore: Williams & Wilkins, 1946. Price, $10.00.

THIS volume represents a new and exceedingly practical approach to the presentation of information for the diagnosis and treatment of disease. Intended for the office, not the library, it has the salient data in readily available form. Good use is made of comparative tables, for instance, in differential diagnosis. Techniques of treatment are described fully and well. Drugs are mentioned by their trade names, with information on their composition.

The first chapter deals with the case history, including its medico-legal aspects. In this, as in succeeding chapters, the author makes use of long and detailed forms for use in history taking and physical diagnosis. The second chapter takes up nutrition and the vitamin deficiencies, and is very well done. Other chapter topics include gastrointestinal diseases, diseases of the heart, peripheral vascular disease, diseases of the kidneys, venereal diseases, diseases of the respiratory system, arthritis and allied conditions, endocrinology for the general practitioner, diseases of allergy, diseases due to intoxications, physical agents and poisons; infectious diseases, tropical diseases of postwar importance, nervous and mental diseases, diagnosis and treatment of common skin disorders, chemotherapy, geriatrics, the care of the ambulatory patient. The chapter on clinical laboratory medicine, by Seward E. Miller, M.D., gives the indications for and the interpretation of results of tests. The chapter on common procedure and diets includes the techniques of thoracentesis, abdominal and pericardial tap, intravenous injections, transfusions, lifting and turning patients, the use of counter-irritants.

Criticisms of minor importance are: inadequate handling of some subjects (disseminated lupus rates only 74 words); a number of consistently misspelled words (for example, Van den Burgh); scarification with a needle is the way *not* to do a cutaneous test for allergy; intracutaneous is a proper term, *not* intradermal; the author uses "regime" when he means "regimen," and "temperature" for "fever;" a 100-grain dose of nitroglycerine will not occur in the next edition.

Commendable are the format with parallel columns instead of the solid page, the many excellent illustrations, and the fine color plates. The book should have a wide appeal.

R. K.

PATHOGENICITY OF CERTAIN SEROLOGICAL TYPES OF B. COLI. By SVEN SJOSTEDT, Univ. of Lund. Pp. 148. Lund, Sweden: Hakan Ohlssons Boktryckeri, 1946. Price not given.

THIS thorough, careful study of 1331 strains of *E. coli* was based upon the serologic classification of Kauffmann, of Knipschildt and of Vahlne. Cultures derived from appendicitis, peritonitis, urinary infections, feces of normal persons, etc., were classified according to their O, K (heat-labile somatic or capsular) and H antigens. Mouse toxicity, hemolysis, necrotizing properties and resistance to phagocytosis were determined. The toxicity of individual capsulated strains of the same serotype are alike in toxicity, irrespective of their origin. Capsulated forms are considerably more toxic than corresponding non-capsulated forms. Hemolysis was associated with certain definite serotypes and there was some correlation between hemolysis and necrotizing ability. In general, cultures which were hemolytic and produced skin necrosis were highly toxic. Strains of the same serotype possessed the same resistance to bactericidal properties of normal human blood, different serotypes varied widely. This resistance had no relation to toxicity. Resistance to phagocytosis was dependent upon a well-developed capsule and phagocytosis was enhanced by the addition of appropriate K serums but not by O serums. There was

little correlation between toxicity and resistance to phagocytosis.

A correlation of clinical histories of cases from which cultures were derived and the properties of the strains isolated led to the conclusion that capsulation, hemolysis and necrotizing capacity are significantly related to toxicity and pathogenicity. Capsular antigens appeared in the urine of patients affected with coli peritonitis. P. E.

THE TREATMENT OF BRONCHIAL ASTHMA. By VINCENT J. DERBES, M.D., Instructor in Medicine and in Preventive Medicine, Tulane University of Louisiana School of Medicine, Director of the Department of Allergy, Ochsner Clinic; and HUGO TRISTRAM ENGELHARDT, M.D., F.A.C.P., Instructor in Clinical Medicine, Baylor University College of Medicine, Houston, Texas, Adjunct in Medicine, Jefferson Davis Hospital; formerly Instructor in Medicine, Tulane University. With chapters by a panel of Contributors. Pp. 466; 61 ills. Philadelphia: J. B. Lippincott, 1946. Price, $8.00.

ENTITLED "The Treatment of Asthma," the book attempts a complete survey of all phases of the subject in an introduction and 23 chapters. The introduction and 15 chapters are by 17 writers other than the 2 authors listed on the title page, who have done only 8 chapters, themselves. This procedure was presumably prompted by the wish to get the views of experts in their several fields. The principle is applicable to textbooks of medicine, but would hardly seem justified to the extent of 19 authors to discuss a single disease. The result in this instance is not a happy one. In general, there is considerable duplication and poor balance. There are some very good chapters such as the history (Major), statistics (Dublin and Marks), anatomy and physiology of the respiratory tract (Burch), pollen survey (Durham), differential diagnosis (Sodeman). Others leave much to be desired, such as the description of the disease, which is quite inadequate; the rôle of foods in asthma, which merely cites all reported instances of foods as allergens but gives no figures on incidence or relative importance, and is uncritical, as in its inclusion of salt as an accepted allergen; the bacterial, epidermal and miscellaneous factors are skimpily handled, as is the chapter on the otolaryngologic aspects. Some of the material is irrelevant, as for instance the inclusion of malaria and amebiasis in the chapter on parasitic agents and asthma, and similarly, the chapter on cardiac asthma could largely be dispensed with. The chapter on psychogenic factors is written with far less insight into matters allergic than most allergists have into psychiatry; those who would challenge this statement should see if the references at the end of the chapter are a fair sample of contemporary opinion. The book is not recommended. R. K.

ACIDOSIS. Clinical Aspects and Treatment With Isotonic Sodium Bicarbonate Solution. By ESBEN KIRK, M.D., Chief Physician, Medical Service, Holstebro District Hospital, Holstebro, Denmark. Pp. 222; 21 ills. Copenhagen: Einar Munksgaard; London: Heinemann, 1946. Price not given.

THE author, at one time a member of Dr. Van Slyke's staff at the Rockefeller Hospital, has been active in encouraging in Denmark the quantitative study of clinical acidosis and its therapy with alkali. The Danish edition of the present work appeared in 1942.

The occurrence of acidosis in some 30 or 40 different clinical conditions is described. These conditions are illustrated with 54 brief case reports which add much to the value of this study. J. A.

QUANTITATIVE CLINICAL CHEMISTRY. INTERPRETATIONS. VOL. I. By JOHN P. PETERS, M.D., M.A., Professor of Internal Medicine, Yale University School of Medicine, and DONALD D. VAN SLYKE, PH.D., D.Sc., Member of the Rockefeller Institute for Medical Research. 2nd ed. Pp. 1041; 62 ills. Baltimore: Williams & Wilkins, 1946. Price, $7.00.

THIS 2nd edition of a well-known book includes in this volume only half of the field of the original "Interpretations," which is now to be expanded to 2 volumes.

The authors have divided their activities; this present volume is mainly the work of Peters, as will be most of the 2nd volume of "Interpretations."

As in the original edition, every statement is based on some specific piece of work for which the reference is given; yet the author

has skillfully woven these separate contributions to our knowledge into a critically evaluated account which gives the reader an integrated picture of the subject being presented: a notable achievement.

Of the approximately 4600 references, about three-fourths seem to the Reviewer to have been published since the date of the 1st edition. It is obvious, therefore, that this volume is essentially a new writing upon current knowledge of the fields covered. These fields include energy metabolism, carbohydrate, lipids and protein metabolism.

Both for the student of the preclinical sciences of physiological chemistry and physiology, and, of course, for the worker in the clinic, this volume will constitute an authoritative text and an indispensable book of reference. J. A.

VOCATIONAL AND PROFESSIONAL MONOGRAPHS—PHYSIOTHERAPY. By THOMAS FRANCIS HENNESSEY, M.D., Dean and Director, Massachusetts School of Physiotherapy, Boston. Pp. 23. Boston: Bellman Publ. Co. Price, $.75

THIS is the 65th in a series of 75 pamphlets which deal with various occupations. Their purpose is to aid individuals in the choice of a career. The author, whose biographical sketch appears in the front of the pamphlet, is a physician who has evidently been well trained in Physical Medicine and is competent to discuss the possibilities of Physical Therapy as a vocation. Throughout, the term Physiotherapy is used instead of the more acceptable designation, Physical Therapy. Non-professional individuals who practice Physical Therapy are referred to as Physical Therapy Technicians and the term Physiotherapist is reserved for physicians who specialize in this field. In accordance with the latest recommendations, the term "technician" should be dropped and replaced by the title Physical Therapist, and physicians who specialize in Physical Medicine should be called Physiatrists.

After a brief account of the history of Physical Therapy and the effects of war upon its development, the present demand for Physical Therapists is stressed. In the discussion of schools for the training of physical therapists several misstatements occur. The American Medical Association within the last 3 years has approved not more than 32 physical therapy schools, and not 80 to 100 as stated. The official statement of the Council of Medical Education and Hospitals of the American Medical Association of April 18, 1946, lists only 21 schools as offering acceptable training. The school of which the author of the pamphlet is Dean and director is not numbered among them. It is stated that graduates from schools of Physical Therapy, when they become 21 years of age automatically become members of "the national association of physiotherapy technicians." More correctly stated, graduates may be elected to the American Physical Therapy Association if their preliminary education and academic record meets the exacting requirements of that organization; to become a member of the American Registry of Physical Therapy Technicians necessitates a separate examination.

The author refers to "Hemo-Irradiation" as "the most outstanding technique of recent years," whereas the procedure is of doubtful clinical value and the instrument required for this technique has never been approved by the Council of Physical Medicine of the American Medical Association.

The pamphlet, which stresses the economic rather than the scientific advantages of Physical Therapy as a career, can hardly be regarded as an authoritative discussion of a field of therapeutics that is constantly growing in importance. G. P.

TREATMENT BY ION TRANSFER. By D. ABRAMOWITSCH, M.D., Physician in Charge of the Physiotherapy Department, Lincoln Hospital, New York City, and B. NEOUSSIKINE, M.D., Tel-Aviv, Palestine. Foreword by RICHARD KOVACS, M.D. Pp. 186. New York: Grune & Stratton, 1946. Price, $4.50.

ION transfer (iontophoresis) is a branch of electrotherapy with which most physicians are unfamiliar. Too often, electrotherapy is thought of as "heat therapy," and the employment of the electric currents, other than for the production of heat, is either looked at askance or disregarded entirely. It is true, that until recent years, there has not been much precise knowledge as to the physics and the clinical applications of the galvanic current; but this ignorance is slowly being dispelled.

This volume answers many questions arising in the physician's mind, concerning ion transfer, and at the same time poses

others for solution. Perhaps the only satisfactory approach to these problems is through the collaboration of the physiologist, the bio-physicist and the clinician in Physical Medicine.

The book is recommended to the medical profession; especially Part 1, which deals with the physical characteristics of the electric current which is used for ion transfer. Part 2 deals with the treatment of individual diseases. Some of the techniques are given in considerable detail, while others are presented somewhat sketchily. The chapters devoted to facial palsy are of especial interest.

For specialists in Physical Medicine and their technical assistants, this stimulating little volume ought to be on the handiest shelf of the library. A large and valuable Bibliography is included. S. H.

HISTORY OF MEDICINE. By DOUGLAS GUTHRIE, M.D., F.R.C.S., Edinburgh. Pp. 448; 72 ills. Philadelphia, London and Montreal: Lippincott, 1946. Price, $6.00.

GUTHRIE's book adds a practical and readable text to the available short histories of medicine. In it the entire development of medical science is integrated in a single continuous story in which a good balance is preserved in the accounts of ancient, medieval and later medicine. The treatment of subjects in the successive chapters is in considerable measure biographical, but with an excellent sense of proportion in the selection of significant advances as represented by the work of distinguished physicians. The detailed story stops with Sir William Osler, whose influence upon medical science is well treated in the final chapter. There is brief mention in a chapter on military and naval surgery of some of the achievements of medicine and surgery in the period "between the wars," including sulfonamide and penicillin treatment and other developments of modern medical science. Medical students will find the chapters on "Specialism and Preventive Medicine" and "Journalism, Bibliography and Medical History" particularly useful for their assessment of these characteristic features of the modern period, which do not receive due attention in many of the other medical historical texts available. The illustrations are excellent. The book is recommended as a useful compendium for those who wish to see medicine in a broad outline, and are not in a position to pursue it in detail through the encyclopedic texts. E. L.

MEDICAL CLINICS OF NORTH AMERICA. May 1946. New York Number. Rheumatic Diseases. Pp. 243. Philadelphia: Saunders, 1946. Price, $16.00 a year.

ARTICLES on rheumatic fever, rheumatoid, menopausal and pneumococcic arthritis, gout and Reiter's syndrome are presented by authorities in the field of rheumatic diseases. In particular, therapy of these varied disease states is emphasized. There are also separate papers on Roentgen irradiation, vitamins, physical medicine and orthopedics in the management of rheumatic diseases. On the whole this section ought to be a valuable adjunct to the practitioner who treats patients afflicted with rheumatic disorders.

A paper entitled "Hypertension Due to Arteriosclerosis, and Its Complications" contains several misstatements which should not be permitted to go unchallenged. The development of hypertension as a consequence of an arteriosclerotic lesion in one of the centers regulating blood pressure is more or less hypothetical and not a statement of fact. It is indeed remarkable that an enema of 16 ounces of water is likely to cleanse the small bowel in preparation for the administration of digitalis by rectum. It is also stated that pulsus alternans can be detected by "taking the diastolic blood pressure and when the lowest point is reached if one pulse beat is heard and the next one falls out, pulsus alternans is present." In the management of congestive failure the Karell diet is said to consist of 1000 to 1500 cc. of milk every 2 hours.

The Reviewer hopes that at least some of these errors are due to careless proofreading. C. F.

NEW BOOKS

A History of Scientific English. The Story of Its Evolution Based on a Study of Biomedical Terminology. By EDMUND ANDREWS, M.D. Foreword by ARNO B. LUCKHARDT, M.D. Pp. 342; 18 figs. New York: Richard R. Smith, 1937. Price, $7.50.

Uterotubal Insufflation. By I. C. RUBIN, M.D., F.A.C.S., Clinical Professor of Gynecology, Coll. of Physicians and Surgeons, Columbia Univ. Pp. 453; 159 ills., 4 in color. St. Louis: C. V. Mosby, 1947. Price, $10.00.

Genetics, Medicine, and Man. By H. J. MULLER, of Indiana Univ., C. C. LITTLE, of the Roscoe B. Jackson Mem. Lab., LAURENCE H. SNYDER, of The Ohio State Univ. Pp. 158; 30 ills. Ithaca: Cornell Univ. Press, 1947. Price, $2.25.

Therapeutic Exercise. By F. H. EWERHARDT, M.D., Ass't. Professor of Physical Medicine, Washington Univ. School of Medicine, and GERTRUDE F. RIDDLE, B.S., R.N., R.P.T., Instructor, School of Physical Medicine, St. Louis Univ. Pp. 152; 8 figs. Phila.: Lea & Febiger, 1937. Price, $2.50.

Die Hormonalen Aspekte des Fortpflanzungsprozesses. By DR. JULES SAMUELS, Chirurg-Frauenarzt. Pp. 152. Amsterdam, Holland: Holdert & Co., N.V., 1946. No price given.

Die Hormonversorgung des Foetus. By DR. JULES SAMUELS, Chirurg-Frauenarzt. Pp. 320. Leiden, Holland: E. J. Brill, 1947. No price given.

Tomorrow's Food. By JAMES RORTY and N. PHILIP NORMAN, M.D. Foreword by STUART CHASE. Pp. 258. New York: Prentice-Hall, 1947. Price, $3.50.

Child Health. "The Practitioner" Handbooks. Edited by ALAN MONCRIEFF, M.D., F.R.C.P., Nuffield Professor of Child Health, Univ. of London, and WILLIAM A. R. THOMSON, M.D. Pp. 254. London: Eyre & Spottiswoode, Ltd., 1947. Price, 14 s, net.

Rehabilitation Through Better Nutrition. By TOM D. SPIES, M.D., Univ. of Cincinnati Studies in Nutrition at the Hillman Hosp., Birmingham, Ala. Pp. 94; 50 figs. Phila.: W. B. Saunders, 1947. Price, $4.00.

NEW EDITIONS

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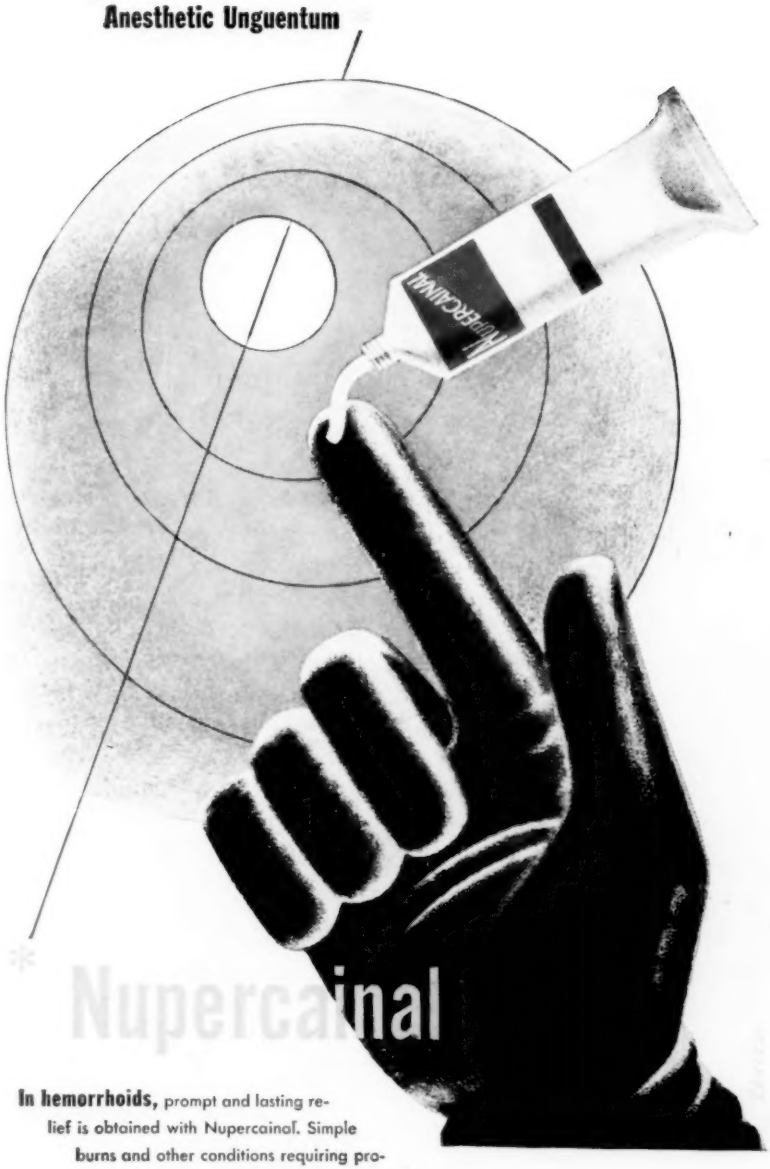

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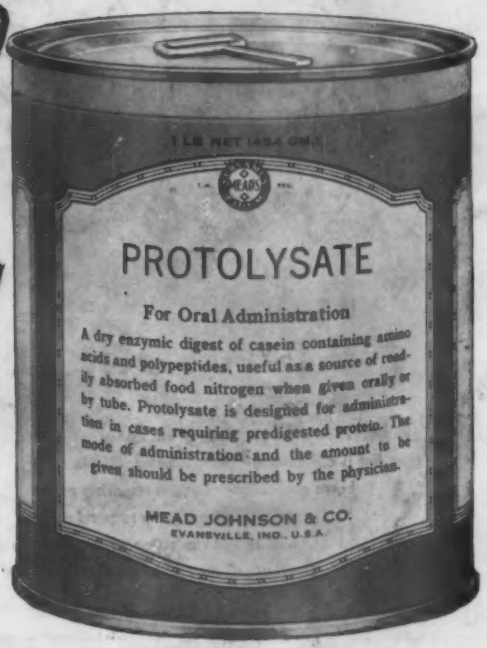
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